WEST Search History

Hide Items | Restore | Clear | Cancel

DATE: Wednesday, June 09, 2004

Hide?	Set Name	Query	Hit Count
		DB=PGPB,USPT,USOC,EPAB,JPAB,DWPI; PLUR=YES; OP=ADJ	
☐	L1	li-X$.in. or kain-S$.in.	3292
☐	L2	fusion protein same (enhanced green fluorescent protein or green fluorescent protein or GFP or EGFP)	2306
☐	L3	l2 and (EGFP or ECFP or EYFP)	461
☐	L4	L3 and (half life same (hours or hr))	21
☐	L5	L3 and (murine ornithine decarboxylase or MODC)	6
☐	L6	L5 and (vector and inducible promoter)	3
☐	L7	L5 and tetracycline	3
☐	L8	l5 and cell line	5
☐	L9	L8 and transfect$	5
☐	L10	l1 and l2	18
☐	L11	fusion protein same (fluorescent protein)	1909
☐	L12	L11 and (half life near (10 or ten near hour))	7
☐	L13	L11 and ((half life) same(10 or ten) near hour)	10
☐	L14	L2 and half life	963
☐	L15	L11 and (half life same (hours or hr))	49

END OF SEARCH HISTORY

Hit List

Clear | Generate Collection | Print | Fwd Refs | Bkwd Refs | Generate OACS

Search Results - Record(s) 1 through 7 of 7 returned.

☐ 1. Document ID: US 20040053388 A1

Using default format because multiple data bases are involved.

L12: Entry 1 of 7 File: PGPB Mar 18, 2004

PGPUB-DOCUMENT-NUMBER: 20040053388
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20040053388 A1

TITLE: Detection of protein conformation using a split ubiquitin reporter system

PUBLICATION-DATE: March 18, 2004

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Eckert, Jorg H.	Witten		DE	
Johnsson, Nils	Eggenstein-Leopoldshafen		DE	
Raquet, Xavier	Xhendelesse		BE	

US-CL-CURRENT: 435/194; 435/320.1, 435/325, 435/69.7, 530/350, 530/399

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWMC | Draw. D

☐ 2. Document ID: US 20040043388 A1

L12: Entry 2 of 7 File: PGPB Mar 4, 2004

PGPUB-DOCUMENT-NUMBER: 20040043388
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20040043388 A1

TITLE: Three hybrid assay system

PUBLICATION-DATE: March 4, 2004

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Come, Jon H.	Cambridge	MA	US	
Becker, Frank	Planegg	MA	DE	
Kley, Nikolai A.	Wellesley		US	
Reichel, Christoph	Planegg		DE	

US-CL-CURRENT: 435/6; 435/7.1, 530/317, 530/350, 536/123, 536/23.1, 540/200, 546/1, 552/200, 552/500, 552/653, 556/118

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw De

☐ 3. Document ID: US 20030165873 A1

L12: Entry 3 of 7 File: PGPB Sep 4, 2003

PGPUB-DOCUMENT-NUMBER: 20030165873
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20030165873 A1

TITLE: Three hybrid assay system

PUBLICATION-DATE: September 4, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Come, Jon H.	Cambridge	MA	US	
Becker, Frank	Planegg	MA	DE	
Kley, Nikolai	Wellesley		US	

US-CL-CURRENT: 435/6; 435/7.1, 530/350, 536/23.1, 536/5, 552/570

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw De

☐ 4. Document ID: US 20020058274 A1

L12: Entry 4 of 7 File: PGPB May 16, 2002

PGPUB-DOCUMENT-NUMBER: 20020058274
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20020058274 A1

TITLE: Rapidly degrading GFP-fusion proteins and methods of use

PUBLICATION-DATE: May 16, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Li, Xianqiang	Palo Alto	CA	US	
Kain, Steve	Belmont	CA	US	

US-CL-CURRENT: 435/6; 435/183, 435/320.1, 435/325, 435/4, 530/350, 536/23.2

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw De

☐ 5. Document ID: US 6306600 B1

L12: Entry 5 of 7 File: USPT Oct 23, 2001

US-PAT-NO: 6306600
DOCUMENT-IDENTIFIER: US 6306600 B1

TITLE: Rapidly degrading GFP-fusion proteins and methods of use

DATE-ISSUED: October 23, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Kain; Steve	Belmont	CA		
Li; Xianqiang	Palo Alto	CA		

US-CL-CURRENT: 435/6; 435/320.1, 435/325, 435/455, 435/69.1, 435/70.1, 530/324, 530/350, 536/23.4, 536/23.5

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw De

☐ 6. Document ID: US 6130313 A

L12: Entry 6 of 7 File: USPT Oct 10, 2000

US-PAT-NO: 6130313
DOCUMENT-IDENTIFIER: US 6130313 A

TITLE: Rapidly degrading GFP-fusion proteins

DATE-ISSUED: October 10, 2000

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Li; Xianqiang	Palo Alto	CA		
Kain; Steve	Belmont	CA		

US-CL-CURRENT: 530/324; 435/7.7, 436/546, 436/800

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw De

☐ 7. Document ID: WO 9954348 A1

L12: Entry 7 of 7 File: EPAB Oct 28, 1999

PUB-NO: WO009954348A1
DOCUMENT-IDENTIFIER: WO 9954348 A1
TITLE: RAPIDLY DEGRADING GFP-FUSION PROTEINS AND METHODS OF USE

PUBN-DATE: October 28, 1999

INVENTOR-INFORMATION:

NAME	COUNTRY
LI, XIANQIANG	
KAIN, STEVE	

ASSIGNEE-INFORMATION:

NAME	COUNTRY
CLONTECH LAB INC	US

APPL-NO: US09824323
APPL-DATE: November 13, 1998

PRIORITY-DATA: US06210298A (April 17, 1998)

INT-CL (IPC): C07 K 14/00; C07 H 21/04; C12 N 15/11; C12 N 15/63; C12 N 1/21; C12 N 15/85; C12 N 15/86
EUR-CL (EPC): C07K014/435; C12N009/88

ABSTRACT:

CHG DATE=19991202 STATUS=O>Green fluorescent protein (GFP) is widely used as a reporter in determining gene expression and protein localization. The present invention provides fusion proteins with a half life of ten hours or less with several embodiments having half lives of 4 hours or less. Such proteins may be constructed by fusing C-terminal amino acids of the degradation domain of mouse ornithine decarboxylase (MODC), which contains a PEST sequence, to the C-terminal end of an enhanced variant of GFP (EGFP). Fluorescence intensity of the fusion protein in transfected cells is similar to that of EGFP, but the fusion protein, unlike EGFP, is unstable in the presence of cycloheximide. Specific mutations in the MODC region have resulted in mutants with varying half lives, useful for a variety of purposes.

Term	Documents
HALF	1523586
HALFS	712
HALVES	193277
LIFE	833472
LIFES	642
LIVE	131563
LIVES	36889
"10"	10993806
10S	5600
TEN	618804
TENS	106270

(L11 AND (HALF LIFE NEAR (10 OR TEN NEAR HOUR))).PGPB,USPT,USOC,EPAB,JPAB,DWPI.	7

There are more results than shown above. Click here to view the entire set.

Display Format: - Change Format

Previous Page Next Page Go to Doc#

Hit List

Clear | Generate Collection | Print | Fwd Refs | Bkwd Refs | Generate OACS

Search Results - Record(s) 1 through 7 of 7 returned.

☐ 1. Document ID: US 20040053388 A1

Using default format because multiple data bases are involved.

L12: Entry 1 of 7 File: PGPB Mar 18, 2004

PGPUB-DOCUMENT-NUMBER: 20040053388
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20040053388 A1

TITLE: Detection of protein conformation using a split ubiquitin reporter system

PUBLICATION-DATE: March 18, 2004

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Eckert, Jorg H.	Witten		DE	
Johnsson, Nils	Eggenstein-Leopoldshafen		DE	
Raquet, Xavier	Xhendelesse		BE	

US-CL-CURRENT: 435/194; 435/320.1, 435/325, 435/69.7, 530/350, 530/399

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWMC | Draw De

☐ 2. Document ID: US 20040043388 A1

L12: Entry 2 of 7 File: PGPB Mar 4, 2004

PGPUB-DOCUMENT-NUMBER: 20040043388
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20040043388 A1

TITLE: Three hybrid assay system

PUBLICATION-DATE: March 4, 2004

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Come, Jon H.	Cambridge	MA	US	
Becker, Frank	Planegg	MA	DE	
Kley, Nikolai A.	Wellesley		US	
Reichel, Christoph	Planegg		DE	

US-CL-CURRENT: 435/6; 435/7.1, 530/317, 530/350, 536/123, 536/23.1, 540/200, 546/1, 552/200, 552/500, 552/653, 556/118

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWMC | Draw De

☐ 3. Document ID: US 20030165873 A1

L12: Entry 3 of 7 File: PGPB Sep 4, 2003

PGPUB-DOCUMENT-NUMBER: 20030165873
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20030165873 A1

TITLE: Three hybrid assay system

PUBLICATION-DATE: September 4, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Come, Jon H.	Cambridge	MA	US	
Becker, Frank	Planegg	MA	DE	
Kley, Nikolai	Wellesley		US	

US-CL-CURRENT: 435/6; 435/7.1, 530/350, 536/23.1, 536/5, 552/570

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWMC | Draw De

☐ 4. Document ID: US 20020058274 A1

L12: Entry 4 of 7 File: PGPB May 16, 2002

PGPUB-DOCUMENT-NUMBER: 20020058274
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20020058274 A1

TITLE: Rapidly degrading GFP-fusion proteins and methods of use

PUBLICATION-DATE: May 16, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Li, Xianqiang	Palo Alto	CA	US	
Kain, Steve	Belmont	CA	US	

US-CL-CURRENT: 435/6; 435/183, 435/320.1, 435/325, 435/4, 530/350, 536/23.2

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWMC | Draw De

☐ 5. Document ID: US 6306600 B1

L12: Entry 5 of 7 File: USPT Oct 23, 2001

US-PAT-NO: 6306600
DOCUMENT-IDENTIFIER: US 6306600 B1

TITLE: Rapidly degrading GFP-fusion proteins and methods of use

DATE-ISSUED: October 23, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Kain; Steve	Belmont	CA		
Li; Xianqiang	Palo Alto	CA		

US-CL-CURRENT: 435/6; 435/320.1, 435/325, 435/455, 435/69.1, 435/70.1, 530/324, 530/350, 536/23.4, 536/23.5

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWMC | Draw De

☐ 6. Document ID: US 6130313 A

L12: Entry 6 of 7 File: USPT Oct 10, 2000

US-PAT-NO: 6130313
DOCUMENT-IDENTIFIER: US 6130313 A

TITLE: Rapidly degrading GFP-fusion proteins

DATE-ISSUED: October 10, 2000

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Li; Xianqiang	Palo Alto	CA		
Kain; Steve	Belmont	CA		

US-CL-CURRENT: 530/324; 435/7.7, 436/546, 436/800

Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWMC | Draw De

☐ 7. Document ID: WO 9954348 A1

L12: Entry 7 of 7 File: EPAB Oct 28, 1999

PUB-NO: WO009954348A1
DOCUMENT-IDENTIFIER: WO 9954348 A1
TITLE: RAPIDLY DEGRADING GFP-FUSION PROTEINS AND METHODS OF USE

PUBN-DATE: October 28, 1999

INVENTOR-INFORMATION:

NAME	COUNTRY
LI, XIANQIANG	
KAIN, STEVE	

ASSIGNEE-INFORMATION:

NAME	COUNTRY
CLONTECH LAB INC	US

APPL-NO: US09824323
APPL-DATE: November 13, 1998

PRIORITY-DATA: US06210298A (April 17, 1998)

INT-CL (IPC): C07 K 14/00; C07 H 21/04; C12 N 15/11; C12 N 15/63; C12 N 1/21; C12 N 15/85; C12 N 15/86
EUR-CL (EPC): C07K014/435; C12N009/88

ABSTRACT:

CHG DATE=19991202 STATUS=O>Green fluorescent protein (GFP) is widely used as a reporter in determining gene expression and protein localization. The present invention provides fusion proteins with a half life of ten hours or less with several embodiments having half lives of 4 hours or less. Such proteins may be constructed by fusing C-terminal amino acids of the degradation domain of mouse ornithine decarboxylase (MODC), which contains a PEST sequence, to the C-terminal end of an enhanced variant of GFP (EGFP). Fluorescence intensity of the fusion protein in transfected cells is similar to that of EGFP, but the fusion protein, unlike EGFP, is unstable in the presence of cycloheximide. Specific mutations in the MODC region have resulted in mutants with varying half lives, useful for a variety of purposes.

Term	Documents
HALF	1523586
HALFS	712
HALVES	193277
LIFE	833472
LIFES	642
LIVE	131563
LIVES	36889
"10"	10993806
10S	5600
TEN	618804
TENS	106270

(L11 AND (HALF LIFE NEAR (10 OR TEN NEAR HOUR))).PGPB,USPT,USOC,EPAB,JPAB,DWPI.	7

There are more results than shown above. Click here to view the entire set.

Display Format: - Change Format

Previous Page Next Page Go to Doc#

Connecting via Winsock to STN

Welcome to STN International! Enter x:x

LOGINID:SSSPTA1655CXW

PASSWORD:
TERMINAL (ENTER 1, 2, 3, OR ?):2

* * * * * * * * * * Welcome to STN International * * * * * * * * * *

NEWS	1		Web Page URLs for STN Seminar Schedule - N. America
NEWS	2		"Ask CAS" for self-help around the clock
NEWS	3	JAN 27	Source of Registration (SR) information in REGISTRY updated and searchable
NEWS	4	JAN 27	A new search aid, the Company Name Thesaurus, available in CA/CAplus
NEWS	5	FEB 05	German (DE) application and patent publication number format changes
NEWS	6	MAR 03	MEDLINE and LMEDLINE reloaded
NEWS	7	MAR 03	MEDLINE file segment of TOXCENTER reloaded
NEWS	8	MAR 03	FRANCEPAT now available on STN
NEWS	9	MAR 29	Pharmaceutical Substances (PS) now available on STN
NEWS	10	MAR 29	WPIFV now available on STN
NEWS	11	MAR 29	New monthly current-awareness alert (SDI) frequency in RAPRA
NEWS	12	APR 26	PROMT: New display field available
NEWS	13	APR 26	IFIPAT/IFIUDB/IFICDB: New super search and display field available
NEWS	14	APR 26	LITALERT now available on STN
NEWS	15	APR 27	NLDB: New search and display fields available
NEWS	16	May 10	PROUSDDR now available on STN
NEWS	17	May 19	PROUSDDR: One FREE connect hour, per account, in both May and June 2004
NEWS	18	May 12	EXTEND option available in structure searching
NEWS	19	May 12	Polymer links for the POLYLINK command completed in REGISTRY
NEWS	20	May 17	FRFULL now available on STN
NEWS	21	May 27	STN User Update to be held June 7 and June 8 at the SLA 2004 Conference
NEWS	22	May 27	New UPM (Update Code Maximum) field for more efficient patent SDIs in CAplus
NEWS	23	May 27	CAplus super roles and document types searchable in REGISTRY
NEWS	24	May 27	Explore APOLLIT with free connect time in June 2004

NEWS EXPRESS	MARCH 31 CURRENT WINDOWS VERSION IS V7.00A, CURRENT MACINTOSH VERSION IS V6.0c(ENG) AND V6.0Jc(JP), AND CURRENT DISCOVER FILE IS DATED 26 APRIL 2004
NEWS HOURS	STN Operating Hours Plus Help Desk Availability
NEWS INTER	General Internet Information
NEWS LOGIN	Welcome Banner and News Items
NEWS PHONE	Direct Dial and Telecommunication Network Access to STN
NEWS WWW	CAS World Wide Web Site (general information)

Enter NEWS followed by the item number or name to see news on that specific topic.

result in loss of user privileges and other penalties.

* * * * * * * * * * * * * * STN Columbus * * * * * * * * * * * * * * *

FILE 'HOME' ENTERED AT 16:42:26 ON 09 JUN 2004

=> file .biotech caplus
COST IN U.S. DOLLARS

	SINCE FILE ENTRY	TOTAL SESSION
FULL ESTIMATED COST	0.21	0.21

FILES 'MEDLINE, BIOTECHDS, EMBASE, BIOSIS, SCISEARCH, CANCERLIT, CAPLUS'
ENTERED AT 16:42:36 ON 09 JUN 2004
ALL COPYRIGHTS AND RESTRICTIONS APPLY. SEE HELP USAGETERMS FOR DETAILS.

7 FILES IN THE FILE LIST

=> s li X?/au or kain S?/au
L1 53940 LI X?/AU OR KAIN S?/AU

=> s fusion protein and (fluorescent protein_
UNMATCHED LEFT PARENTHESIS 'AND (FLUORESCEN'
The number of right parentheses in a query must be equal to the
number of left parentheses.

=> s fusion protein and (fluorescent protein)
L2 7357 FUSION PROTEIN AND (FLUORESCENT PROTEIN)

=> s fusion protein and (enhanc## green fluorescent protein or GFP or green
fluorescent protein or EGFP)
L3 9189 FUSION PROTEIN AND (ENHANC## GREEN FLUORESCENT PROTEIN OR GFP
OR GREEN FLUORESCENT PROTEIN OR EGFP)

=> s fusion protein and (ECFP or EYFP or EGFP)
L4 1417 FUSION PROTEIN AND (ECFP OR EYFP OR EGFP)

=> s (l2 or l3 or l4) and half life
L5 66 (L2 OR L3 OR L4) AND HALF LIFE

=> dup rem l5
PROCESSING COMPLETED FOR L5
L6 25 DUP REM L5 (41 DUPLICATES REMOVED)

=> d ibib abs l6 1-25

L6 ANSWER 1 OF 25 CAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 2004:252739 CAPLUS
DOCUMENT NUMBER: 140:282429
TITLE: Fusion proteins comprising reporter proteins with reduced **half-life** and use for detecting gene expression
INVENTOR(S): Zdanovsky, Alexey; Zdanovskaia, Marina; Ma, Dongping; Wood, Keith V.; Almond, Brian D.; Wood, Monika G.
PATENT ASSIGNEE(S): Promega Corporation, USA
SOURCE: PCT Int. Appl., 91 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2004025264	A2	20040325	WO 2003-US28939	20030916

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD

RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG

PRIORITY APPLN. INFO.: US 2002-411070P P 20020916
US 2002-412268P P 20020920

AB The present invention provides a fusion polypeptide comprising a protein of interest which has a reduced **half-life** of expression, and a nucleic acid mol. encoding the fusion polypeptide, are provided. Specifically, the invention provides improved gene products comprising reporter proteins, such as a luciferase, **GFP**, chloramphenicol acetyltransferase, beta-glucuronidase or beta-galactosidase, with substantially reduced or decreased half-lives of expression, which are useful to determine or detect gene expression, e.g., up- or down-regulation, to monitor promoter activity, to reduce cytotoxicity, and to localize proteins.

L6 ANSWER 2 OF 25 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
ACCESSION NUMBER: 2004-00800 BIOTECHDS
TITLE: Novel library of fusion nucleic acids each of which has fused first and second nucleic acids encoding scaffold protein and library peptide having alpha helical biasing sequence, respectively, useful in screening methods; DNA library and **fusion protein** for use in disease therapy and gene therapy
AUTHOR: ANDERSON D; PEELLE B R; BOGENBERGER J M
PATENT ASSIGNEE: RIGEL PHARM INC
PATENT INFO: US 2003143562 31 Jul 2003
APPLICATION INFO: US 2002-177725 20 Jun 2002
PRIORITY INFO: US 2002-177725 20 Jun 2002; US 1998-169015 8 Oct 1998
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2003-829786 [77]
AN 2004-00800 BIOTECHDS
AB DERWENT ABSTRACT:

NOVELTY - A library (I) of fusion nucleic acids, where each fusion nucleic acid comprises a first nucleic acid (N1), encoding a scaffold protein sequence; and a second nucleic acid (N2), encoding a library peptide sequence comprising an alpha helical biasing sequence; where N1 is fused to N2, is new.

DETAILED DESCRIPTION - A library of fusion nucleic acids, where each fusion nucleic acid comprises a first nucleic acid (N1), encoding a scaffold protein sequence; and a second nucleic acid (N2), encoding a library peptide sequence comprising an alpha helical biasing sequence; where N1 is fused to N2. Optionally, the library comprises fusion nucleic acids each comprising N1, a second nucleic acid (N2a) encoding library peptide sequence and third nucleic acid (N3) encoding a nucleating sequence, where N1 is fused to N2a, and N2a is fused to N3. INDEPENDENT CLAIMS are also included for the following: (1) a library of retroviral vectors comprising (I); (2) a library of host (II) cells comprising (I); (3) a library of host cells comprising the library of retroviral vectors; (4) a library of fusion polypeptides, where each polypeptide comprises a scaffold protein, and a library peptide comprising an alpha helical biasing sequence; and (5) a library of fusion polypeptides, where each polypeptide comprises a scaffold protein, and a library peptide, and a nucleating sequence.

BIOTECHNOLOGY - Preferred library: N1 is fused to the 5' or 3' end

of N2, or N2 is inserted into N1. N2 comprises nucleic acid sequence encoding random amino acids. The library peptide sequence replaces at least one residue of the scaffold protein sequence. N1 encodes a **green fluorescent protein** (**GFP**) and
N2 is inserted into N1 within positions encoding amino acids 130-135, 154-159, 172-175, 188-193, or 208-216. The **GFP** protein is from Aequorea victoria, where the library peptide sequence replaces the C-Terminal amino acids Leu-Gly-Met-Asp-Glu-Leu-Tyr-Lys of the Aequorea victoria **GFP**; or Renilla mulleri where the library peptide sequence replaces the C-Terminal amino acids Leu-Gly-Ser-Leu-His-Glu-Trp-Val of the Renilla mulleri **GFP**, or Ptilosarcus. N1 optionally encodes a beta-lactamase protein, a dihydrofolate reductase (DHFR) protein, a beta-galactosidase protein, or a luciferase protein. The fusion nucleic acid further comprises a fourth nucleic acid encoding a linker peptide. The fourth nucleic acid is positioned between the first nucleic acid and the second nucleic acid. The fusion nucleic acid further comprises a fifth nucleic acid encoding a second linker peptide. N2a comprises nucleic acid sequence derived from cDNA. N3 is fused to the 5' end of N2a. Preferred Host cell: The host cells are mammalian host cells. Preferred Method: The library of cells containing (I) is provided by transfecting the cells with a library of retroviral vectors comprising the library of fusion nucleic acids.

USE - (II) is useful for screening (M1) for bioactive peptides conferring a particular phenotype which involves providing (II), where each fusion nucleic acid encodes a **fusion protein**,
providing conditions under which the fusion proteins are expressed, and assaying the cells for the phenotype (claimed). (M1) is useful for screening bioactive peptides conferring a change in specific phenotype such as cell morphology, cell growth, cell viability, adhesion to substrates or other cells, and cellular density; changes in the expression of one or more RNAs, proteins, lipids, hormones, cytokines, or other molecules; changes in the equilibrium state (i.e., **half-life**) or one or more RNAs, protein, lipids, hormones, cytokines, or other molecules; etc. The bioactive peptide identified by above mentioned method is used to generate more candidate peptides. The bioactive peptide or the bioactive nucleic acid encoding it is used to identify target molecules, i.e., the molecules with which the bioactive peptide interacts. The bioactive peptide is used to pull out target molecules. The peptide, when expressed in bacteria and purified, can be used as a probe against a bacterial cDNA expression library made from mRNA of the target cell type. Or, peptides can be used as bait in either yeast or mammalian two or three hybrid systems. The peptide(s) can be combined with other pharmacologic activators to study the epistatic relationships of signal transduction pathways in question. (M1) is also is useful in cancer applications. Random libraries can be introduced into any tumor cell (primary or cultured), and peptides identified which by themselves induce apoptosis, cell death, loss of cell division or decreased cell growth. The method is also useful for screening of bioactive peptides which restore the constitutive function of the brca-1 or brca-2 genes, and other tumor suppressor genes important in breast cancer such as the adenomatous polyposis coli gene (APC) and the Drosophila discs-large gene (DIg), which are components of cell-cell junctions. The methods are useful in cardiovascular applications, neurobiology applications, bone biology applications, skin biology applications, cosmeceutical applications, endocrinology applications, infectious disease applications, drug toxicities and drug resistance applications, immunobiology, inflammation, and allergic response applications, and biotechnology applications.

ADVANTAGE - The peptide library can easily be monitored, both for its presence within cells and its quantity. The expression of structurally biased libraries generate elevated cellular concentration of peptides having a given structural bias and thus increase the hit rate for targets that bind such structures.

EXAMPLE - No relevant example is given. (110 pages)

L6 ANSWER 3 OF 25 MEDLINE on STN DUPLICATE 1
ACCESSION NUMBER: 2003456094 MEDLINE
DOCUMENT NUMBER: PubMed ID: 14517305
TITLE: The COOH-terminal domain of wild-type Cot regulates its stability and kinase specific activity.
AUTHOR: Gandara Maria Luisa; Lopez Pilar; Hernando Raquel; Castano Jose G; Alemany Susana
CORPORATE SOURCE: Instituto de Investigaciones Biomedicas Alberto Sols, Consejo Superior de Investigaciones Cientificas, Facultad Medicina, Universidad Autonoma de Madrid, 28029 Madrid, Spain.
SOURCE: Molecular and cellular biology, (2003 Oct) 23 (20) 7377-90. Journal code: 8109087. ISSN: 0270-7306.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200311
ENTRY DATE: Entered STN: 20031001
Last Updated on STN: 20031218
Entered Medline: 20031125

AB Cot, initially identified as an oncogene in a truncated form, is a mitogen-activated protein kinase kinase kinase implicated in cellular activation and proliferation. Here, we show that this truncation of Cot results in a 10-fold increase in its overall kinase activity through two different mechanisms. Truncated Cot protein exhibits a lower turnover rate (**half-life**, 95 min) than wild-type Cot (**half-life**, 35 min). The degradation of wild-type and truncated Cot can be specifically inhibited by proteasome inhibitors in situ. The 20S proteasome also degrades wild-type Cot more efficiently than the truncated protein. Furthermore, the amino acid 435 to 457 region within the wild-type Cot COOH-terminal domain confers instability when transferred to the yellow **fluorescent protein** and targets this **fusion protein** to degradation via the proteasome. On the other hand, the kinase specific activity of wild-type Cot is 3.8-fold lower than that of truncated Cot, and it appears that the last 43 amino acids of the wild-type Cot COOH-terminal domain are those responsible for this inhibition of kinase activity. In conclusion, these data demonstrate that the oncogenic activity of truncated Cot is the result of its prolonged **half-life** and its higher kinase specific activity with respect to wild-type Cot.

L6 ANSWER 4 OF 25 MEDLINE on STN DUPLICATE 2
ACCESSION NUMBER: 2003137444 MEDLINE
DOCUMENT NUMBER: PubMed ID: 12651910
TITLE: The serum-inducible protein kinase Snk is a G1 phase polo-like kinase that is inhibited by the calcium- and integrin-binding protein CIB.
AUTHOR: Ma Sheng; Liu Mei-Ann; Yuan Yi-Lu O; Erikson Raymond L
CORPORATE SOURCE: The Biological Laboratories, Department of Molecular and Cellular Biology, Harvard University, Cambridge, MA 02138, USA.. shengma@fas.harvard.edu
CONTRACT NUMBER: CA42580 (NCI)
GM59172 (NIGMS)
SOURCE: Molecular cancer research : MCR, (2003 Mar) 1 (5) 376-84. Journal code: 101150042. ISSN: 1541-7786.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200310
ENTRY DATE: Entered STN: 20030325
Last Updated on STN: 20031009

Entered Medline: 20031008

AB Identified as an immediate-early transcript, the serum-inducible kinase Snk bears sequence homology with the polo-like kinases. Endogenous Snk was detected in early G(1) in NIH 3T3 cells, and nascent Snk showed a **half-life** of about 15 min. The kinase activity of endogenous Snk was detected in G(1). Substitution of Thr-236 with a glutamate residue increased Snk kinase activity by about 10-fold, whereas substitution of Lys-108 abolished its kinase activity. Disrupting the polo-box did not significantly change Snk kinase activity. A **GFP** -C-Snk **fusion protein** showed polo-box-dependent localization to the microtubule organizing center, and ectopic expression of Snk in COS-7 cells induced changes in cell morphology, depending on Snk kinase activity and the polo-box. The capacity of Snk to induce morphological changes was inhibited by the calcium- and integrin-binding protein CIB. CIB co-immunoprecipitated with Snk and inhibited the kinase activity of Snk, suggesting that CIB is a negative regulator for Snk kinase activity.

L6 ANSWER 5 OF 25 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
ACCESSION NUMBER: 2003-07912 BIOTECHDS
TITLE: Detecting protein interactions in a host cell comprises providing a host cell having nucleic acid coding sequences encoding a bait or a prey **fusion protein** and detecting the subcellular location of the prey protein within the host cell;
vector-mediated gene transfer and expression in host cell for protein interaction detection
AUTHOR: MCKEON F; YANG A
PATENT ASSIGNEE: HARVARD COLLEGE
PATENT INFO: WO 2002086450 31 Oct 2002
APPLICATION INFO: WO 2002-US13008 22 Apr 2002
PRIORITY INFO: US 2001-285509 20 Apr 2001; US 2001-285509 20 Apr 2001
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2003-093165 [08]
AN 2003-07912 BIOTECHDS
AB DERWENT ABSTRACT:
NOVELTY - Detecting protein interactions in a host cell, comprises providing a host cell comprising nucleic acid coding sequences encoding a bait **fusion protein** (B) or a prey **fusion protein** (P), and detecting the subcellular location of the (P) within the host cell, where accumulation of (P) at the same subcellular pattern as (B) indicates that (P) is associated in a complex with (B).
DETAILED DESCRIPTION - Detecting protein interactions in a host cell, comprises providing a host cell comprising nucleic acid coding sequences encoding a bait **fusion protein** (B) or a prey **fusion protein** (P), and detecting the subcellular location of the (P) within the host cell, where accumulation of (P) at the same subcellular pattern as (B) indicates that (P) is associated in a complex with (B). In detail the method comprises: (a) providing a host cell including: (i) a first nucleic acid coding sequence encoding a bait **fusion protein** having a bait polypeptide sequence fused to a targeting domain that targets the bait **fusion protein** to a subcellular location within the host cell; (ii) a second nucleic acid coding sequence encoding a prey **fusion protein** having a prey polypeptide sequence fused to one or more detection domains detected in a transcriptionally-independent manner in the host cell to determine if the prey **fusion protein** is associated in a complex with the bait **fusion protein** or not; (b) detecting the subcellular location of the prey protein within the host cell, where accumulation of the prey fusion at the same subcellular pattern as the bait fusion indicates that the prey **fusion protein** is associated in a complex with the bait **fusion protein**.

INDEPENDENT CLAIMS are also included for the following: (1) a kit for detecting protein interactions, comprising: (a) a first expression construct including a coding sequence for a targeting domain and a ligation site flanking an end of the targeting domain coding sequence for ligating a coding sequence of a bait polypeptide sequence in frame with the targeting domain coding sequence to produce a bait **fusion protein**, the first expression construct operably linked to a transcriptional regulatory element; and (b) a second expression construct including a coding sequence for a detection domain and a ligation site flanking an end of the detection domain coding sequence for ligating a coding sequence of a prey polypeptide sequence in frame with the detection domain coding sequence to produce a prey **fusion protein**, the second expression construct operably linked to a transcriptional regulatory element, where the targeting domain localizes the bait **fusion protein** to a subcellular location within a host cell; (2) a method for conducting a drug discovery business, comprising: (a) identifying a protein complex for which an agent that inhibits or potentiates the formation or activity of the complex is desired; (b) generating a drug screening assay for identifying agents that inhibit or potentiate the formation or activity of the complex; (c) conducting animal toxicity profiles on the agent identified in (b), or its analog; (d) manufacturing a pharmaceutical preparation of the agent having a suitable animal toxicity profile; and (e) marketing the preparation to healthcare providers; (3) a method for conducting a bioinformatics business, comprising: (a) identifying networks of protein complexes; and (b) generating a database including information identifying interactions of different proteins in a signal pathway and information identifying the proteins; and (4) a system for analyzing protein complexes in cells, comprising a flow cytometer for analyzing cells, and determining if a fluorescent signal is dispersed in a cell or localized to kinetochore structures.

BIOTECHNOLOGY - Preferred Method: In detecting protein interactions in a host cell, the targeting domain localizes the bait **fusion protein** to a subcellular compartment or organelle, such as the nucleus, nucleoli, telomeres, kinetochores, nuclear envelope, chromosomes, chromatin, cytoplasm, endoplasmic reticulum, Golgi, centrosome, transgolgi network, cytoplasmic vesicles, mitochondria, secretory vesicles, lysosome, plasma membrane, intracellular membrane vesicles, nuclear membranes, synapses or basolaternal membranes. The targeting domain preferably localizes the bait **fusion protein** to a kinetochore structure. The targeting domain includes an amino acid sequence from a protein selected from CENP-A, CENP-B, CENP-C, CENP-E, CENP-F, Bub1, Bub3, MAD3L and MAD2, or their homologous sequence. The targeting domain comprises at least amino acids 373-943 of the human CENP-C sequence or a sequence that is at least 80% identical to the CENP-C sequence. The targeting domain associates with the kinetochore structure with a dissociation constant (kd) of 1 mm or less. The domain also localizes the bait **fusion protein** to the nuclear envelope, where the targeting domain comprises an amino acid sequence from a protein selected from lamin A, lamin B, lamin C, emerins and porins, or its portion. The detection domain is a fluorescent polypeptide sequence or a luminescent polypeptide sequence. The domain is all or a fluorescent portion of a **green fluorescent protein** sequence. The localization of the prey fusion proteins can be determined within 120 minutes of expression. The prey **fusion protein** further includes an instability sequence that renders the prey **fusion protein** with a shorter intracellular **half-life** when not associated in complexes with the bait **fusion protein** relative to when it is. The instability sequence comprises at least amino acids 249-323 of the human CENP-C sequence or its homologous sequence. The bait or the prey fusion proteins includes a rescue sequence such as His6 tag, myc tag, flu tag, lacZ, GST, or Strep tag I or II. The bait and/or prey **fusion protein** include(s) an oligomerization domain.

The coding sequences for the bait and prey fusion proteins are operably linked to the same or different transcriptional regulatory sequence(s), and are provided on the same or different expression vector(s). At least one of the coding sequences are provided as part of an integrative or an episomal vector. The vector is a retroviral vector. The coding sequences are provided as part of a vector which includes a recovery element. The host cell is a mammalian, preferably human, cell. The subcellular location of the prey protein is determined in the presence of a test agent contacted with the cell. The test agent is a small organic molecule which includes a portion that is predetermined to bind to one of the bait or prey **fusion protein**, and a test portion which is being tested for binding to the other **fusion protein**. The method is carried out consecutively or simultaneously for a library of at least 100 different test agents having varied test portions amongst members of the library. The ability of the test compound to inhibit or potentiate the association of the prey **fusion protein** in a complex with the bait **fusion protein** is determined. The identity of the test agents in the library is also determined. Determining the presence of the subcellular location of the prey protein also comprises the step of formulating a pharmaceutical preparation including one or more compounds identified as inhibitors or potentiators of the association of the prey **fusion protein** in a complex with the bait **fusion protein**, or their analogs. The subcellular location of the prey protein is determined after induction of the host cell with an agent that causes post-translational modification of proteins in the host cell. The subcellular location is determined using flow cytometry analysis or microscopy. Detecting protein interactions in a host cell, further comprises: (a) providing a host cell culture, the cells of which include the first and second nucleic acid coding sequences cited above, where the culture is a variegated mixture of cells containing different prey polypeptide sequences and/or different bait polypeptide sequences; (b) selecting cells from the culture in which the prey **fusion protein** is localized in the cell in the same subcellular pattern as the bait **fusion protein**; and (c) identifying the sequence of the bait and prey fusion proteins from the selected cells. The method for conducting a drug discovery business, comprises identifying the protein complex mediated by post-translational modification, and generating a drug screening assay for identifying agents that inhibit or potentiate the post-translational modification and effect the formation of the protein complex. Preferred System: The system for analyzing protein complexes in cells includes a microprocessor for comparing the flow spectra of cells and distinguishing between a diffuse pattern of fluorescence in the cells and a kinetochore-localized pattern. The system further comprises a microscope having a mounted camera for analyzing cells in a field of vision of the microscope, and a microprocessor for processing images obtained from the camera and determining if a fluorescent signal is dispersed in a cell or localized to kinetochore structures. The system also comprises a cell picking robot controlled by the microprocessor, and isolates cells which the microprocessor has determined to have a fluorescent signal localized to kinetochore structures.

USE - The method is useful in identifying protein interactions in mammalian host cells, and in screening for compounds capable of inhibiting or potentiating the protein-protein interaction. The mammalian two-hybrid system is useful for analyzing protein complexes in cells by flow cytometry analysis or microscopy.

EXAMPLE - No relevant example given.(89 pages)

L6 ANSWER 6 OF 25 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
ACCESSION NUMBER: 2002-09655 BIOTECHDS
TITLE: Selecting RNA and/or DNA molecule binding fluorophore (FP) and increases fluorescence intensity, comprises selecting population of molecules which bind FP, contacting it with FP

and selecting molecules based on fluorescence;
RNA and DNA aptamer, sense, antisense molecule, **fluorescent protein**, bacterium, fungus and insect cell useful in gene therapy, DNA location and quantitation and RNA interaction

AUTHOR: DAVIS J H
PATENT ASSIGNEE: GEN HOSPITAL CORP
PATENT INFO: WO 2002006827 24 Jan 2002
APPLICATION INFO: WO 2000-US21304 5 Jul 2000
PRIORITY INFO: US 2000-215973 5 Jul 2000
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2002-179821 [23]
AN 2002-09655 BIOTECHDS
AB DERWENT ABSTRACT:

NOVELTY - Selecting (M1) an RNA and/or DNA molecule (NA) which binds a fluorophore (FP) and increases the fluorescence intensity (FI), by providing a population of candidate NA molecules, selecting NA which bind FP, contacting selected NA molecules with FP and selecting NA which, upon binding FP, increases its FI, is new.

DETAILED DESCRIPTION - An INDEPENDENT CLAIM is also included for a population of nucleic acids having two loops separated by a base-paired helix or flanked by a base-paired one side and a hairpin on the other side, or having a pseudoknot structure, where a nucleic acid has a non-naturally-occurring polynucleotide sequence.

BIOTECHNOLOGY - Preferred Method: In M1, FP is preferably immobilized and contacting NA with FP is preferably performed by incubating cells with FP, where the cells contain one or more NA which bind FP, and selecting NA which increases FI is performed by sorting the cells based on FI and recovering DNA coding sequence of NA or recovering NA from the sorted cells. The method further comprises repeating all of the above steps.

USE - M1 is useful for selecting NA molecule which binds a FP and increases FI, where the selected NA molecule called aptamers may be used in: (1) determining the presence, location or quantity of a desired NA in a cell or in vitro sample by: (a) expressing a fusion NA comprising desired NA covalently linked to NA aptamer; (b) contacting the cell or the sample with FP, where the aptamer binds to FP and increases its FI; and (c) visualizing or measuring the fluorescence of FP; (2) determining whether a compound is capable of modulating the transcription of an desired RNA, by performing step (a) and (b), and measuring FI in the presence and absence of the compound, where the compound is determined to be an inhibitor or inducer of the transcription and is preferably a member of a library of at least 50 compounds, all of which are simultaneously contacted with the cell or sample; (3) determining whether a compound modulates the **half-life** of desired RNA by performing the steps of (2), where the compound is determined to decrease the **half-life** it is reduces FI and increases **half-life** if it increases FI and the compound degrades, induces or suppresses the degradation of desired RNA. The aptamers is also utilized for determining whether an antisense nucleic acid (ANA) binds a target RNA in a cell or in vitro sample by: (1) (A): (a) expressing in the cell or sample a fusion RNA comprising the target RNA covalently linked to an RNA aptamer; (b) contacting the cell or cell with either ANA and FP or quencher or with FP alone, where FI of FP is decreased by the quencher; and (c) measuring FI in the presence and absence of ANA, where ANA is determined to bind target RNA if ANA decreases FI; (2) (B): performing step (a), contacting the cell or sample with a FP (FP1) and ANA covalently linked to another FP (FP2) (or nucleic acid aptamer which binds to FP2) or with FP1 alone, where APM binds to FP1 and increases FI, and the emission wavelength of FP1 is different from FP2, where emission wavelength of FP2 induces the fluorescence of FP1, and measuring FI of FP1 in the presence and absence of ANA, where ANA is determined to bind target RNA if ANA increases FI of FP1; (3) (C):

performing step (a), contacting the cell or sample with FP and ANA covalently linked to nucleic acid aptamer which binds a quencher or FP alone, where RNA aptamer binds to FP and increases its FI and the aptamer binds to the quencher, which decreases the FI of FP, and measuring FI of FP in the presence and absence of ANA. The aptamer is also utilized for determining whether a first RNA is co-localized with the second desired RNA in a cell or in vitro sample. The method comprises expressing the first fusion RNA (or fusion RNA) comprising RNA covalently linked to RNA aptamer which binds to FP1, and a second fusion RNA (or **fusion protein**) comprising another RNA covalently linked to different RNA aptamer which binds to FP2, in two different cell or sample, contacting the cell with FP1 and FP2 (or FP1) and visualizing the fluorescence of FP1 and FP2 (or FP1), where first desired RNA is determined to co-localize with second RNA (or desired protein) of the FI is detected proximal to fluorescence of FP2 (or protein), where the protein has intrinsic fluorescence or luminescence and is preferably a **green fluorescent protein**. The above method also utilized for determining whether a desired RNA interacts with another desired RNA or protein in a cell or in vitro sample. The cells utilized in all of the above methods is a prokaryotic cell preferably gram-negative or gram-positive bacterial cell, or eukaryotic cell which include yeast, Caenorhabditis, Xenopus, Drosophila, zebrafish, squid, plant, mammalian or human cell, embryonic cell. The containing step of the methods is performed by incubating the cell or the sample with FP or by injecting FP into the cell (all claimed).

EXAMPLE - To design candidate DNA molecules, pools (of about 10 to the power of 16) of candidate DNAs were generated, where candidate molecules with a loop flanked by a strong base-paired helix and hairpin and a candidate nucleic acids having designed to have a pseudoknot were preferred. The fluorophore has a low fluorescence intensity when unbound in aqueous solution and a high fluorescence intensity when bound or otherwise sequestered from water, was synthesized with a linkers that is terminated by an activated group, such as maleimide, isothiocyanate, succinate, or sulfhydyl group. Once the fluorophore was immobilized, the matrix containing the fluorophore was then poured into a column. Selection for molecules that bind to small molecules connected to columns in this manner can produce sequences that fold around the ligand, leaving the region connected to the linker exposed Dieckmann et al., RNA 2:628, 1996 and Zimmermann et al., Nature Struct. Biol. 4:644, 1997. To increase the likelihood of selecting DNA molecules that no only bind the fluorophore but also increases its fluorescent intensity, possibly by sequestering the fluorophore from water, several different linker or connecting groups should be utilized to allow binding of the DNA molecules in several different orientations. The DNA molecules that bind the fluorophore may be selected using a modification of the Systematic Evolution of Ligands by Exponential Enrichment (SELEX) method U.S. Patent Number 5270163 and Ellington et al., Nature 346:818-822, 1990. The DNA molecules can dissociate from the immobilized fluorophore and bind the fluorophore in solution instead of rebinding the immobilized fluorophore. The eluted DNA may be amplified using polymerase chain reaction (PCR) for use in additional rounds of selection. Repeating the selection several times may enrich the population for DNA molecules that bind the fluorophore. (70 pages)

L6 ANSWER 7 OF 25 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
ACCESSION NUMBER: 2002-09918 BIOTECHDS
TITLE: Producing a protein of interest, e.g., a pharmaceutically active protein, comprises expressing a polynucleotide fusion construct in a plastid and producing a **fusion protein** comprising the protein of interest;
tobacco transgenic plant construction via plasmid and Agrobacterium sp.-mediated gene transfer for interferon, human interferon-gamma, beta-glucuronidase and **green fluorescent protein**

production
AUTHOR: REDDY V S; SADHU L
PATENT ASSIGNEE: INT CENT GENETIC ENG and BIOTECHNOLOGY
PATENT INFO: WO 2002006497 24 Jan 2002
APPLICATION INFO: WO 2000-EP8132 14 Jul 2000
PRIORITY INFO: GB 2000-17397 14 Jul 2000
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2002-171810 [22]
AN 2002-09918 BIOTECHDS
AB DERWENT ABSTRACT:

NOVELTY - Producing (M1) a protein of interest involves allowing a polynucleotide fusion construct (FC) to be expressed in a plastid to produce a **fusion protein** comprising the protein of interest, where FC comprises a polynucleotide coding sequence of the protein of interest operably linked to a polynucleotide coding sequence of a **fusion protein** partner.

DETAILED DESCRIPTION - In M1, the **fusion protein** expressed has a greater **half-life** than the individually expressed protein of interest, is resistant to internal cleavage in vivo, and comprises a cleavage site. INDEPENDENT CLAIMS are also included for the following: (1) obtaining (M2) a transplastomic plastid, by transforming a plastome within a plastid by a FC; (2) obtaining (M3) a transplastomic cell, by transforming a plastid within a cell by M2; (3) obtaining (M4) a homotransplastomic cell, by obtaining transplastomic cells by M3 and selecting for the presence of the transplastosome; (4) obtaining (M5) a first-generation transplastomic or homotransplastomic plant, by regenerating a transplastomic or homotransplastomic plant cell obtainable by M3 or M4 to give a transplastomic or homotransplastomic plant; (5) obtaining (M6) a transplastomic or homotransplastomic plant seed, by obtaining a transplastomic or homotransplastomic plant seed from a transplastomic or homotransplastomic plant obtainable by M5; (6) obtaining (M7) a transplastomic or homotransplastomic progeny plant by obtaining a second-generation transplastomic or homotransplastomic progeny plant from a first-generation transplastomic or homotransplastomic plant by M5, and optionally obtaining transplastomic or homotransplastomic plants of one or more further generations from the second-generation progeny plants thus obtained; (7) use of FC or a polynucleotide encoding a **fusion protein** partner to increase the stability of the recombinantly expressed protein of interest in a plastid; (8) a transplastosome (I), a transplastomic or homotransplastomic plastid, a transplastomic or homotransplastomic plant cell, transplastomic or homotransplastomic callus, a transplastomic or homotransplastomic first-generation plant, transplastomic or homotransplastomic plant seed or progeny plant obtained by M2-M7 or comprising FC; (9) a **fusion protein** or protein of interest obtained by M1 or from a cell or plant obtained by M3-M7; (10) obtaining (M8) crop product by harvesting crop product from a cell or plant obtained by M3-M7, and optionally further processing the harvested product; and (11) a crop product obtained by M8.

BIOTECHNOLOGY - Preferred Method: In M1, the plastid is a chloroplast. The accumulation of the **fusion protein** as a percentage of the total cell protein in a cell comprising the plastid in M1 is from 10-1000 fold higher than the accumulation of the individually expressed protein of interest. The **fusion protein** partner has a scorable property, and comprises beta-glucuronidase (GUS) or **Green fluorescent protein** (**GFP**), or a variant or fragment of either that has GUS or **GFP** activity. The N-terminal of the **fusion protein** partner is fused to the C-terminal of the polypeptide of interest. The cleavage site of the **fusion protein** is IEGR. The **fusion protein** comprises a purification tag, which comprises a sequence which facilitates affinity purification.

The purification tag is preferably His-tag. The purification tag is retained at a terminus of the fusion partner following in vitro cleavage of the cleavage site. M1 further comprises obtaining a sample comprising expressed **fusion protein**, enriching the obtained sample in the **fusion protein** by fractionating the sample comprising the **fusion protein** and selecting the fraction or fractions enriched in the **fusion protein** by detecting the scorable property of the **fusion protein** partner, to provide the sample enriched in the **fusion protein**, recovering the **fusion protein** from a sample by a affinity-based technique that uses the purification tag of the **fusion protein** partner, and cleaving the **fusion protein** in vitro to release the protein of interest, where the cleavage has a fidelity of at least 95%. M7 comprises obtaining a transplastomic or homotransplastomic seed from a first-generation transplastomic or homotransplastomic plant obtainable by M5, then obtaining a second-generation transplastomic or homotransplastomic progeny plant from the seed, propagating clonally the first-generation transplastomic or homotransplastomic plant to give a second-generation transplastomic or homotransplastomic progeny plant, and/or crossing the first-generation transplastomic or homotransplastomic plant with another plant to give a second-generation transplastomic or homotransplastomic progeny plant, and optionally, obtaining transplastomic or homotransplastomic progeny plants of one or more further generations from the second-generation transplastomic or homotransplastomic progeny plant thus obtained. Preferred Transplastosome: In (I), the polynucleotide fusion construct is integrated into the plastosome.

USE - M1 is useful for producing a protein of interest which comprises a human protein, or its biologically active variant or fragment, a pharmaceutically active protein, an interferon, or its biologically active variant or fragment, or a human interferon-gamma (hIFN-gamma), or its biologically active variant or fragment (claimed). The methods are useful for production of transgenic plants. The protein of interest obtained by M1 is useful for the manufacture of a medicament for treating a disease condition.

EXAMPLE - Transformation and regeneration of stable transgenic plants was as follows: The Agrobacterium mediated transformation method was followed for nuclear transformation of tobacco with pBI121 and pBIIFNG binary vectors under kanamycin selection. Particle bombardment of leaf tissue was used for chloroplast transformation under spectinomycin selection using DNA of vectors pVSR326, p326IFNG, and pGUSIFNG. Tobacco (Nicotiana tabacum) was transformed using particle delivery system PDS1000 according to the method described by (Svab and Maliga 1993). In brief, vector DNA coated on to tungsten particles was bombarded on the abaxial side of a tobacco leaf placed on RMOP medium, a modified Murashige and Skoog (MS) medium. Transformed shoots were selected on RMOP medium containing 500 mg/l spectinomycin dihydrochloride. 3 additional cycles of regeneration on spectinomycin containing RMOP medium was carried out to obtain homotransplastomic plastid containing plants. The Agrobacterium strain LBA 4404 containing vector pBIIFNG/pBI121 was used for nuclear transformation following a leaf disc method. Although the vector DNA was randomly delivered into leaf cells in the particle bombardment method, the selectable aadA was expected to express and confer resistance to spectinomycin only when it entered the chloroplasts due to the specificity of the rrn promoter. Substantially, homotransplastomic lines were established by repeating regeneration process three times from the leaf tissues of primary transformants under spectinomycin selection. Nt. BI121-1, Nt. BIIFNG-1/2, Nt. VSR326-37, Nt. 326IFNG-1/2 and Nt. GUSIFNG-1 plants transformed with vectors pBI121, pB1IFNG, pVSR326, p326IFNG, and pGUSIFNG, respectively, were subjected to molecular analysis to confirm the transgenic nature of regenerated plants and the expression of recombinant interferon-gamma (IFN-gamma). (92 pages)

L6 ANSWER 8 OF 25 MEDLINE on STN DUPLICATE 3
ACCESSION NUMBER: 2002364493 MEDLINE
DOCUMENT NUMBER: PubMed ID: 12112006
TITLE: Characterization of stably transfected **fusion protein GFP**-estrogen receptor-alpha in MCF-7 human breast cancer cells.
AUTHOR: Zhao Helen; Hart Laura L; Keller Ulrike; Holth Laurel T; Davie James R
CORPORATE SOURCE: Manitoba Institute of Cell Biology, University of Manitoba, 675 McDermot Avenue, Winnipeg, Canada R3E 0V9.
SOURCE: Journal of cellular biochemistry, (2002) 86 (2) 365-75. Journal code: 8205768. ISSN: 0730-2312.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200212
ENTRY DATE: Entered STN: 20020712
Last Updated on STN: 20021218
Entered Medline: 20021213

AB Tagging hormone receptors with the **green fluorescent protein** (**GFP**) has increased our knowledge of ligand dependent sub-cellular trafficking of hormone receptors. However, the effect of the tagged hormone receptor expression on the corresponding wild type hormone receptor and endogenous gene expression has not been investigated. In this study, we constructed a MCF-7 cell line stably expressing **GFP**-tagged human estrogen receptor-alpha (ER) under control of the tetracycline-on system to determine the effect of **GFP**-ER expression on cell proliferation and expression of endogenous ER and hormone-responsive genes. Further, the inducible system was applied to determine the ligand dependent turnover rates of **GFP**-ER protein and mRNA. Our results demonstrate that **GFP** -ER expression did not affect cell cycling. Independent of ligand, **GFP**-ER markedly reduced the level of endogenous ER mRNA and protein, suggesting that ER negatively autoregulates its expression. Cisplatin cross-linking studies showed that **GFP**-ER is associated with nuclear DNA in situ, suggesting that **GFP**-ER is partially replacing ER at estrogen response elements. Furthermore, **GFP**-ER expression did not affect the estradiol induced temporal expression of hormone responsive genes c-myc and pS2.

L6 ANSWER 9 OF 25 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
ACCESSION NUMBER: 2002-06722 BIOTECHDS
TITLE: New **fusion protein** comprising a modified bioluminescent system with a fluorescent molecule covalently linked with a photoprotein, useful for monitoring calcium fluxes or for detecting electrical activity in a group of neural cells;
plasmid pGIA-pG5A-mediated gene transfer and expression in host cell for recombinant mutant **green fluorescent protein** and aequorin **fusion protein** production
AUTHOR: BAUBET V; LE MOUELLIC H; BRULET P
PATENT ASSIGNEE: INST PASTEUR; CENT NAT RECH SCI
PATENT INFO: WO 2001092300 6 Dec 2001
APPLICATION INFO: WO 2000-EP7057 1 Jun 2000
PRIORITY INFO: US 2000-255111 14 Dec 2000
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2002-139605 [18]
AN 2002-06722 BIOTECHDS
AB DERWENT ABSTRACT:

NOVELTY - A **fusion protein** for energy transfer from aequorin to **green fluorescent protein** by Chemiluminescence Resonance Energy Transfer (CRET), which comprises a fluorescent molecule covalently linked with a photoprotein, is new. The **fusion protein** comprises the formula (I).

DETAILED DESCRIPTION - The **fusion protein** comprises: **GFP** - LINKER - AEQ (I) **GFP** = **green fluorescent protein**; AEQ = aequorin; and LINKER = a polypeptide of 4-63 amino acids. INDEPENDENT CLAIMS are also included for the following: (1) a purified polypeptide having the sequence comprising 432, 441, 450, 468, 477 or 906 amino acids (designated GA, G1A, G2A, G4A, G5A and SeG5A, respectively) fully defined in the specification; (2) a purified polynucleotide having the sequence comprising 1296, 1323, 1350, 1404, 1431 or 1308 bp (which corresponds to GA, G1A, G2A, G4A, G5A and SeG5A, respectively) fully defined in the specification; (3) a polynucleotide linker having the polynucleotide sequence comprising 15, 42, 69, 123 or 150 bp fully defined in the specification; (4) a peptidic linker of at least 5 amino acids and comprising the sequence having 5 (SGLRS), 14 (SGGSGSGGGSGLRS), 23, 41 or 50 amino acids fully defined in the specification; (5) a modified bioluminescent system comprising two bioluminescent proteins and any of the peptide linker cited above; (6) a composition comprising a purified polypeptide, where the composition has the functional characteristics to bind calcium ions and to permit a measurable energy, the energy depending of the quantity of calcium bound and of the quantity of polypeptides in the composition in absence of any light excitation; (7) a kit for measuring the transfer of energy in vivo or in vitro and containing at least one of the polypeptides or polynucleotides, and the reagents necessary for visualizing or detecting the transfer in presence or in absence of a molecule of interest; (8) a polynucleotide encoding the **fusion protein**; (9) cultures as deposited at the C.N.C.M. and containing plasmids Number I-2507, Number I-2508, Number I-2509, Number I-2510, Number I-2511, Number I-2512 or Number I-2513; (10) screening in vivo a change in a physical, chemical, biochemical or biological condition; (11) screening of a product leading to a change in a physical, chemical, biochemical or biological condition in vivo, comprising: (a) administering to a vertebrate a pharmaceutical medium comprising the composition in presence or in absence of a molecule of interest to be tested; (b) detecting the energy produced in presence of the composition; and(c) optionally, measuring the effective concentration of the molecule of interest necessary for the detection of the energy in step (b); and (12) screening in vitro a molecule capable of modulating the energy in the composition comprising: (a) providing in a biological sample the composition or in a reaction system containing the molecule to be tested; (b) detecting a modulation of the energy by comparison with a control sample containing the composition without the molecule to be tested; and (c) optionally, determining the effective minimal concentration of the molecule capable of inhibiting or increasing the energy transfer of the composition.

BIOTECHNOLOGY - Preferred Bioluminescent System: The polynucleotide linker has the function after translation to approach a donor site to an acceptor site in optimal conditions to permit a direct transfer of energy by Chemiluminescence Resonance Energy Transfer (CRET) in the purified polypeptide. The peptide linker has the function to approach a donor site to an acceptor site in optimal conditions to permit a direct transfer of energy by chemiluminescence in the purified polypeptide. The peptide linker also has the function to approach a donor site to an acceptor site in optimal conditions to permit a direct transfer of energy in the presence of the purified polypeptide. The peptide linker has the capacity to stabilize a modified bioluminescent system in vivo and/or in vitro. Preferably, the two bioluminescent proteins comprise at least an aequorin protein. The modified bioluminescent system comprises the following

constituents: aequorin protein and a **GFP** protein. The linker comprises 14-50 amino acids, where the linker comprises the following amino acids: (Gly Gly Ser Gly Ser Gly Gly Gln Ser)n, where n is 1-5. The **fusion protein** for energy transfer from aequorin to **green fluorescent protein** by CRET following activation of the aequorin in the presence of Ca++, where the **fusion protein** has an affinity for Ca++ ions and a **half-life** of at least 24 hours. The **fusion protein** further comprises a peptide signal sequence for targeting the **fusion protein** to a cell or to a subcellular compartment. The purified polypeptide is the purified polypeptide GA, G1A, G2A, G4A, G5A or SeG5A, the modified bioluminescent system or the **fusion protein**. Preferred Method: Screening in vivo a change in a physical, chemical, biochemical or biological condition comprises: (a) administering to a mammal the composition; (b) detecting whether the light is produced; and (c) optionally measuring the ionic concentration of calcium flux. The method may also involve: (a) adding into a reaction system the composition containing an analyte of interest in presence or in absence of a molecule of interest to be tested; and (b) visualizing the emission of energy produced in step (a).

USE - The **fusion protein** is useful as a bioluminescent Ca+ reporter at the single cell level. The **fusion protein**, composition or bioluminescent system is useful for monitoring calcium fluxes in real time. This is particularly useful for understanding the development, the plasticity, and the functioning of the central nervous system. The **fusion protein** comprising the bioluminescent system is useful for detecting electrical activity in a group of neural cells, for making it possible to complete the phenotype study of mutants, or for observing the calcium activity in a population of connected cells, for example in a neural network.

EXAMPLE - All the constructs were made in the pEGFP-C1 vector. The AGFP gene was codon-optimized for maximal expression in mammalian cells. It also contained 2 mutations in the chromophore, F64L and S65T, which modified the excitation spectra and enhanced fluorescence intensity. The aequorin coding sequence has been fused in frame at the 3' end of the **EGFP** gene in the BgIII/SaII sites of pEGFP-C1. Seven codons were modified for a better expression in mammalian cells by means of site-directed mutagenesis using PCR with overlap extension. Then, complementary oligonucleotides, 5'-CCGGCGGGAGCGGATCCGGCGGCCAGT-3' and 5'-CCGGACTGGCCGCCGGATCCGCTCCCG-3' were inserted at the BspEI site in the 15 bp sequence between **GFP** and aequorin. Conservation of the BspEI site at only one end allowed sequential addition of one to five linker sequences (pGIA-pG5A). (58 pages)

L6 ANSWER 10 OF 25 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN

ACCESSION NUMBER: 2001:566551 BIOSIS
DOCUMENT NUMBER: PREV200100566551
TITLE: Rapidly degrading **GFP**-fusion proteins and methods of use.
AUTHOR(S): Kain, Steve [Inventor]; Li, Xianqiang [Inventor]
CORPORATE SOURCE: ASSIGNEE: Clontech Laboratories, Inc.
PATENT INFORMATION: US 6306600 October 23, 2001
SOURCE: Official Gazette of the United States Patent and Trademark Office Patents, (Oct. 23, 2001) Vol. 1251, No. 4. e-file. CODEN: OGUPE7. ISSN: 0098-1133.
DOCUMENT TYPE: Patent
LANGUAGE: English
ENTRY DATE: Entered STN: 12 Dec 2001
Last Updated on STN: 25 Feb 2002

AB **Green fluorescent protein** (**GFP**) is widely used as a reporter in determining gene expression and protein localization. The present invention provides fusion proteins with a **half life** of ten hours or less with several embodiments having half lives of 4 hours or less. Such proteins may be constructed by

fusing C-terminal amino acids of the degradation domain of mouse ornithine decarboxylase (MODC), which contains a PEST sequence, to the C-terminal end of an enhanced variant of **GFP** (**EGFP**). Fluorescence intensity of the **fusion protein** in transfected cells is similar to that of **EGFP**, but the **fusion protein**, unlike **EGFP**, is unstable in the presence of cycloheximide. Specific mutations in the MODC region have resulted in mutants with varying half lives, useful for a variety of purposes.

L6 ANSWER 11 OF 25 MEDLINE on STN DUPLICATE 4
ACCESSION NUMBER: 2001275781 MEDLINE
DOCUMENT NUMBER: PubMed ID: 11359927
TITLE: Dynamics of immature secretory granules: role of cytoskeletal elements during transport, cortical restriction, and F-actin-dependent tethering.
AUTHOR: Rudolf R; Salm T; Rustom A; Gerdes H H
CORPORATE SOURCE: Department of Neurobiology, Interdisciplinary Center for Neuroscience, University of Heidelberg, Im Neuenheimer Feld 364, D-69120 Heidelberg, Germany.
SOURCE: Molecular biology of the cell, (2001 May) 12 (5) 1353-65. Journal code: 9201390. ISSN: 1059-1524.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200109
ENTRY DATE: Entered STN: 20010917
Last Updated on STN: 20010917
Entered Medline: 20010913

AB Secretory granules store neuropeptides and hormones and exhibit regulated exocytosis upon appropriate cellular stimulation. They are generated in the trans-Golgi network as immature secretory granules, short-lived vesicular intermediates, which undergo a complex and poorly understood maturation process. Due to their short **half-life** and low abundance, real-time studies of immature secretory granules have not been previously possible. We describe here a pulse/chase-like system based on the expression of a human chromogranin B-**GFP** **fusion protein** in neuroendocrine PC12 cells, which permits direct visualization of the budding of immature secretory granules and their dynamics during maturation. Live cell imaging revealed that newly formed immature secretory granules are transported in a direct and microtubule-dependent manner within a few seconds to the cell periphery. Our data suggest that the cooperative action of microtubules and actin filaments restricts immature secretory granules to the F-actin-rich cell cortex, where they move randomly and mature completely within a few hours. During this maturation period, secretory granules segregate into pools of different motility. In a late phase of maturation, 60% of secretory granules were found to be immobile and about half of these underwent F-actin-dependent tethering.

L6 ANSWER 12 OF 25 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 2001:427921 BIOSIS
DOCUMENT NUMBER: PREV200100427921
TITLE: Determination of **GFP**-estrogen receptor **fusion protein half-life** in MCF-7 breast cancer cells.
AUTHOR(S): Hart, Laura L. [Reprint author]; Davie, James R. [Reprint author]
CORPORATE SOURCE: Manitoba Institute of Cell Biology, University of Manitoba, Winnipeg, MB, R3E 0V9, Canada
SOURCE: Biochemistry and Cell Biology, (2001) Vol. 79, No. 3, pp. 382. print.
Meeting Info.: 22nd Annual West Coast Chromatin and

Chromosomes Conference. Pacific Grove, California, USA. December 07-10, 2000.
CODEN: BCBIEQ. ISSN: 0829-8211.
DOCUMENT TYPE: Conference; (Meeting)
Conference; Abstract; (Meeting Abstract)
LANGUAGE: English
ENTRY DATE: Entered STN: 12 Sep 2001
Last Updated on STN: 22 Feb 2002

L6 ANSWER 13 OF 25 MEDLINE on STN DUPLICATE 5
ACCESSION NUMBER: 2001300614 MEDLINE
DOCUMENT NUMBER: PubMed ID: 11164310
TITLE: The basal turnover of yeast branched-chain amino acid permease Bap2p requires its C-terminal tail.
AUTHOR: Omura F; Kodama Y; Ashikari T
CORPORATE SOURCE: Institute for Fundamental Research, Suntory Ltd., 1-1-1, Wakayamadai, Shimamoto-cho, Mishima-gun, 618-8503, Osaka, Japan.. fumihiko_omura@suntory.co.jp
SOURCE: FEMS microbiology letters, (2001 Jan 15) 194 (2) 207-14.
Journal code: 7705721. ISSN: 0378-1097.
PUB. COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200105
ENTRY DATE: Entered STN: 20010604
Last Updated on STN: 20010604
Entered Medline: 20010531

AB The branched-chain amino acid permease Bap2p is a transport system for leucine, isoleucine, and valine in Saccharomyces cerevisiae, and its synthesis is regulated transcriptionally. However, the downregulation mechanisms of Bap2p have not been established. Here we demonstrate that the C-terminal region of Bap2p plays a pivotal role in its basal turnover. Truncation of the C-terminal 29 residues caused the stabilization and accumulation in the plasma membrane of Bap2p. Furthermore, when the Bap2p C-terminal region was fused to **green fluorescent protein**, the **fusion protein** localized to the plasma membrane, suggesting the existence of a possible degradation-related acceptor site for the C-terminal tail of Bap2p.

L6 ANSWER 14 OF 25 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
ACCESSION NUMBER: 2001-01398 BIOTECHDS
TITLE: New rapidly degrading **green fluorescent protein** (**GFP**)-fusion proteins, useful in a variety of research applications, e.g. as a reporter in determining gene expression and protein localization; constructed by of fusing C-terminus amino acids of the degradation domain mouse ornithine-decarboxylase
AUTHOR: Li X; Kain S
PATENT ASSIGNEE: Clontech-Lab.
LOCATION: Palo Alto, CA, USA.
PATENT INFO: US 6130313 10 Oct 2000
APPLICATION INFO: US 1998-62102 17 Apr 1998
PRIORITY INFO: US 1998-62102 17 Apr 1998
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2000-655680 [63]
AN 2001-01398 BIOTECHDS
AB A **fusion protein** comprising **green fluorescent protein** (**GFP**) and a PEST sequence is claimed. The protein has a **half-life** of no more than ten hours. The **fusion protein** is useful in research applications, e.g. as a genetic reporter for analyzing transcriptional regulation and/or cis-acting regulatory element, as a

tool for identifying and dissecting degradation domains of short-lived proteins, or in drug screening assays. The method provides a rapid turnover or destabilized **GFP** that can be used in research applications where prior method GFPs cannot. A rapid turnover **GFP** permits development of a stable cell line, which expresses the **GFP** gene since toxic levels of **GFP** are avoided, as the **GFP** protein is quickly degraded. Also disclosed are: an isolated DNA molecule encoding a **GFP fusion protein** with a **half life** decreased from that of wild type **GFP**; a vector capable of expressing the isolated DNA molecule; a method of producing a stable cell line that expresses **GFP** by transfecting cells with the vector; and a method of labelling cells with a transient **GFP** reporter. (18pp)

L6 ANSWER 15 OF 25 MEDLINE on STN
ACCESSION NUMBER: 2001106010 MEDLINE
DOCUMENT NUMBER: PubMed ID: 10991948
TITLE: Ubiquitin-mediated proteolysis of a short-lived regulatory protein depends on its cellular localization.
AUTHOR: Lenk U; Sommer T
CORPORATE SOURCE: Max-Delbruck-Centrum fur Molekulare Medizin, Robert-Rossle-Strasse 10, Berlin 13092, Germany.
SOURCE: Journal of biological chemistry, (2000 Dec 15) 275 (50) 39403-10.
Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200102
ENTRY DATE: Entered STN: 20010322
Last Updated on STN: 20030202
Entered Medline: 20010208

AB In this study we demonstrate that the Deg1 degradation signal of the transcriptional repressor Matalpha2 confers compartment-specific turnover to a reporter protein. Rapid degradation of a Deg1-containing **fusion protein** is observed only when the reporter is efficiently imported into the nucleus. In contrast, a reporter that is constantly exported from the nucleus exhibits an extended **half-life**. Furthermore, nuclear import functions are crucial for both Deg1-induced degradation as well as for the turnover of the endogenous Matalpha2 protein. The conjugation of ubiquitin to a Deg1-containing reporter protein is abrogated in mutants affected in nuclear import. Obviously, the Deg1 signal initiates rapid proteolysis within the nucleoplasm, whereas in the cytosol it mediates turnover via a slower pathway. In both pathways the ubiquitin-conjugating enzymes Ubc6p/Ubc7p play a pivotal role. These observations imply that both the cellular targeting of a substrate and the compartment-specific activity of components of the ubiquitin-proteasome system define the **half-life** of naturally short-lived proteins.

L6 ANSWER 16 OF 25 MEDLINE on STN DUPLICATE 7
ACCESSION NUMBER: 2000422566 MEDLINE
DOCUMENT NUMBER: PubMed ID: 10922050
TITLE: The N terminus of microsomal delta 9 stearoyl-CoA desaturase contains the sequence determinant for its rapid degradation.
AUTHOR: Mziaut H; Korza G; Ozols J
CORPORATE SOURCE: Department of Biochemistry, University of Connecticut Health Center, Farmington, CT 06030-3305, USA.
CONTRACT NUMBER: RO1 GM-26351 (NIGMS)
SOURCE: Proceedings of the National Academy of Sciences of the United States of America, (2000 Aug 1) 97 (16) 8883-8.
Journal code: 7505876. ISSN: 0027-8424.

PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200009
ENTRY DATE: Entered STN: 20000915
Last Updated on STN: 20000915
Entered Medline: 20000905

AB Stearoyl-CoA desaturase (SCD) is a key regulator of membrane fluidity, turns over rapidly, and represents a model for selective degradation of short-lived proteins of the endoplasmic reticulum (ER). The mechanism whereby specific ER proteins are targeted for degradation in the midst of stable proteins coexisting in the same membrane is unknown. To investigate the intracellular fate of SCD and to identify the determinants involved in the rapid turnover of SCD, we created chimeras of SCD tagged at the C terminus with the **green fluorescent protein** (**GFP**). The fusion proteins were expressed in Chinese hamster ovary cells and exhibited an ER localization. Unlike native **GFP**, the SCD-**GFP** construct was unstable and had a **half life** of a few hours. Truncated fusion proteins consisting of residues 27-358 and 45-358 of SCD linked to the N terminus of **GFP** were stable. To investigate the general applicability of the N terminus of SCD in the destabilization of proteins, we fused residues 1-33 of SCD to the N terminus of **GFP**. The resulting chimera was extremely short lived. To investigate the effect of membrane sidedness on the **fusion protein** degradation, we attached a lumenal targeting signal to the N terminus of SCD 1-33-**GFP**. The construct was localized to the lumen of ER and was metabolically stable, indicating that SCD degradation signal functions on the cytosolic rather than the lumenal side of the ER. These results demonstrate that the N-terminal segment of some 30 residues of SCD constitutes a motif responsible for the rapid degradation of SCD.

L6 ANSWER 17 OF 25 CAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 2000:785228 CAPLUS
DOCUMENT NUMBER: 134:82937
TITLE: Destabilized **green fluorescent protein** for monitoring dynamic changes in yeast gene expression with flow cytometry
AUTHOR(S): Mateus, Carolina; Avery, Simon V.
CORPORATE SOURCE: Department of Biology, Georgia State University, Atlanta, GA, 30303, USA
SOURCE: Yeast (2000), 16(14), 1313-1323
CODEN: YESTE3; ISSN: 0749-503X
PUBLISHER: John Wiley & Sons Ltd.
DOCUMENT TYPE: Journal
LANGUAGE: English

AB **Green fluorescent protein** (**GFP**) has many advantages as a reporter mol., but its stability makes it unsuitable for monitoring dynamic changes in gene expression, among other applications. Destabilized GFPs have been developed for bacterial and mammalian systems to counter this problem. Here, we extend such advances to the yeast model. We fused the PEST-rich 178 carboxyl-terminal residues of the G1 cyclin Cln2 to the C terminus of yEGFP3 (a yeast- and FACS-optimized **GFP** variant), creating yEGFP3-Cln2PEST. We tested the hybrid protein after integrating modules harboring the yEGFP3 or yEGFP3-CLN2PEST ORFs into the Saccharomyces cerevisiae genome. YEGFP3-Cln2PEST had a markedly shorter **half-life** (t1/2) than yEGFP3; inhibition of protein synthesis with cycloheximide lead to a rapid decline in **GFP** content and fluorescence (t1/2 .apprx.30 min) in cells expressing yEGFP3-Cln2PEST, whereas these parameters were quite stable in yEGFP3-expressing cells (t1/2 .apprx.7 h). We placed yEGFP3-CLN2PEST under the control of the CUP1 promoter, which is induced only transiently by copper. This transience was readily

discernible with yEGFP3-Cln2PEST, whereas yEGFP3 reported only on CUP1 switch-on, albeit more slowly than vEGFP3-Cln2PEST. Cell cycle-regulated transcriptional activation/inactivation of the CLN2 promoter was also discernible with yEGFP3-Cln2PEST, using cultures that were previously synchronized with nocodazole. In comparison to CLN2, expression from the ACT1 promoter was stable after release from nocodazole. We also applied a novel flow-cytometric technique for cell cycle anal. with asynchronous cultures. The marked periodicities of CLN2 and CLB2 (mitotic cyclin) transcription were readily evident from cellular yEGFP3-Cln2PEST levels with this non-perturbing approach. The results represent the first reported successful destabilization of a yeast-**GFP**. This new construct expands the range of **GFP** applications open to yeast workers.

REFERENCE COUNT: 38 THERE ARE 38 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L6 ANSWER 18 OF 25 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
ACCESSION NUMBER: 2001-01377 BIOTECHDS
TITLE: A ubiquitin-based tagging system for controlled modulation of protein stability;
beta-lactamase and **green fluorescent protein** recombinant **fusion protein** expression in Jurkat cell culture and protein stabilization
AUTHOR: Stack J H; Whitney M; Rodems S M; Pollok B A
CORPORATE SOURCE: Aurora-Biosciences
LOCATION: Aurora Biosciences Corporation, 11010 N. Torreyana Road, San Diego, CA 92121, USA.
Email: stackj@aurorabio.com
SOURCE: Nat.Biotechnol.; (2000) 18, 12, 1298-302
CODEN: NABIF
ISSN: 1087-0156
DOCUMENT TYPE: Journal
LANGUAGE: English
AN 2001-01377 BIOTECHDS
AB A lot of biotechnology applications require expression of exogenous proteins in a predictable and controllable way. A key determinant of the intracellular concentration of a given protein is its stability or **half-life**. A versatile and reliable system has been developed for producing short **half-life** forms of proteins expressed in mammal cells. The system consists of a series of destabilization domains comprising varying numbers of a mutant form of ubiquitin (Ub-G76V) that cannot be cleaved by ubiquitin-hydrolases. Increasing the numbers of Ub-G76V moieties within the destabilization domain (by site-directed mutagenesis using vector plasmid pcDNA3-Bla-**GFP**) gives a graded reduction in protein **half-life** and steady-state levels when fused to heterologous reporter proteins (e.g. **green fluorescent protein**) as well as cellular proteins. Cells expressing a destabilized Escherichia coli beta-lactamase (EC-3.5.2.6) reporter act as a robust, high throughput screening-compatible assay for proteasome activity within cells. (25 ref)

L6 ANSWER 19 OF 25 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
ACCESSION NUMBER: 2000-01528 BIOTECHDS
TITLE: New **fusion protein** useful for studying cell localization, for studying cell lineage and for assaying activation or deactivation of transcriptional or translational elements;
green fluorescent protein and mouse ornithine-decarboxylase **fusion protein** expression in host cell
AUTHOR: Li X; Kain S
PATENT ASSIGNEE: Clontech-Lab.

LOCATION: Palo Alto, CA, USA.
PATENT INFO: WO 9954348 28 Oct 1999
APPLICATION INFO: WO 1998-US24323 13 Nov 1998
PRIORITY INFO: US 1998-62102 17 Apr 1998
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2000-013225 [01]
AN 2000-01528 BIOTECHDS
AB A **fusion protein** containing a **fluorescent protein** related to **green fluorescent protein** and mouse ornithine-decraboxylase (EC-4.1.1.17) degradation domain C-terminal amino acids and having a **half-life** of no more than 10 hr, is new. Also claimed are: a DNA molecule encoding the **fusion protein**; a vector; a stable cell line that expresses a **fluorescent protein**; and production of the cell line. The protein can be used for assaying activation or deactivation of transcriptional or translational elements with a transient fluorescent reporter protein. The protein can be used to study cell lineage. It can also be linked with different enhancer elements and used to monitor biological processes e.g. heat response, response to cyclic-AMP etc. (49pp)

L6 ANSWER 20 OF 25 MEDLINE on STN DUPLICATE 9
ACCESSION NUMBER: 1999340063 MEDLINE
DOCUMENT NUMBER: PubMed ID: 10409681
TITLE: Characterization of NFkappaB activation by detection of **green fluorescent protein**-tagged IkappaB degradation in living cells.
AUTHOR: Li X; Fang Y; Zhao X; Jiang X; Duong T; Kain S R
CORPORATE SOURCE: CLONTECH Laboratories, Inc., Palo Alto, California 94303, USA.. xqli@clontech.com
CONTRACT NUMBER: 1R43GM58288-01 (NIGMS)
SOURCE: Journal of biological chemistry, (1999 Jul 23) 274 (30) 21244-50.
Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199908
ENTRY DATE: Entered STN: 19990910
Last Updated on STN: 20000303
Entered Medline: 19990826
AB Activation of the transcription factor NFkappaB requires rapid degradation of its inhibitor, IkappaBalpha. To facilitate the study of IkappaBalpha degradation, we fused IkappaBalpha protein to **enhanced green fluorescent protein** to construct IkappaBalpha-**enhanced green fluorescent protein** (IG). We demonstrated by both flow cytometry and Western blot analysis that the **half-life** of IG in the presence of human tumor necrosis factor (TNF) alpha is approximately 5 min, which is similar to the **half-life** of native IkappaBalpha. The degradation coincided with NFkappaB translocation from the cytoplasm to the nucleus and NFkappaB-mediated induction of transcription. Phorbol 12-myristate 13-acetate (PMA), but not forskolin, also induces degradation of IG **fusion protein**. The **half-life** of IG in the presence of PMA is approximately 15 min, longer than when induced with TNFalpha. Co-treatment with TNFalpha and PMA did not result in a synergistic effect on IG degradation, although they stimulate different kinases in two different signaling pathways. Degradation of IG was inhibited by mutations at serine residues 32 and 36, which are the target sites of the phosphorylation modification that initiates degradation of IkappaBalpha. We also demonstrated that basal degradation of IG in the presence of cycloheximide is inhibited by such mutations,

suggesting that basal degradation of IkappaBalpha also requires phosphorylation as the signal for degradation. Finally, we showed that the rate of TNFalpha-induced degradation of IG remains almost constant throughout the cell cycle, except at the mitotic phase, in which IG degrades more slowly.

L6 ANSWER 21 OF 25 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
ACCESSION NUMBER: 2000-00992 BIOTECHDS
TITLE: Attenuation of **green fluorescent protein half-life** in mammalian cells;
protein engineering for fluorescence protein stabilization
AUTHOR: Corish P; Tyler-Smith C
CORPORATE SOURCE: Univ.Oxford
LOCATION: CRC Chromosome Molecular Biology Group, Department of Biochemistry, University of Oxford, South Parks Road, OX1 3QU, UK.
Email: chris@bioch.ox.ac.uk
SOURCE: Protein Eng.; (1999) 12, 12, 1035-40
CODEN: PRENE9
ISSN: 0269-2139
DOCUMENT TYPE: Journal
LANGUAGE: English
AN 2000-00992 BIOTECHDS
AB The **half-life** of the **green fluorescent protein** (**GFP**) was determined biochemically in cultured mouse LA-9 cells. The wild-type protein was found to be stable with a **half-life** of about 26 hr, but could be destabilized by the addition of putative proteolytic signal sequences derived from proteins with shorter half-lives. A C-terminal fusion of a PEST sequence from the mouse ornithine-decarboxylase (EC-4.1.1.17) gene reduced the **half-life** to 9.8 hr, giving a **GFP** variant suitable for the study of dynamic cellular processes. In an N-terminal fusion containing the mouse cyclin B1 destruction box, it was reduced to 5.8 hr with most degradation occurring at metaphase. The combination of both sequences gave a similar **GFP half-life** of 5.5 hr. The stability of the marker protein can be controlled in a predetermined way by addition of the appropriate proteolytic signals. Protein engineering can only be performed at the ends of the molecule otherwise the barrel structure of **GFP** is lost and the molecule looses its fluorescence. (32 ref)

L6 ANSWER 22 OF 25 MEDLINE on STN DUPLICATE 11
ACCESSION NUMBER: 1999115928 MEDLINE
DOCUMENT NUMBER: PubMed ID: 9914420
TITLE: Green fluorescent proteins with short half-lives as reporters in Dictyostelium discoideum.
AUTHOR: Deichsel H; Friedel S; Detterbeck A; Coyne C; Hamker U; MacWilliams H K
CORPORATE SOURCE: Zoologisches Institut, Ludwig-Maximilians-Universitat, Luisenstrasse 14, D-80333 Munchen, Germany.
SOURCE: Development genes and evolution, (1999 Jan) 209 (1) 63-8.
Journal code: 9613264. ISSN: 0949-944X.
PUB. COUNTRY: GERMANY: Germany, Federal Republic of
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199903
ENTRY DATE: Entered STN: 19990324
Last Updated on STN: 19990324
Entered Medline: 19990309
AB We describe two modifications of the popular reporter **green fluorescent protein** (**GFP**) which have short half-lives in our system, the cellular slime mould Dictyostelium

discoideum. One of these bears an N-terminal ubiquitin; this **GFP** was originally planned to be a substrate of the "N-end-rule" pathway, but deubiquitination does not seem to occur, and a degradation by the UFD (ubiquitin-fusion-degradation pathway seems more probable. The protein **half-life** is about 3-5 h. The second construct has an N-terminus derived from the L11 ribosomal protein; it is transported to the nucleus and broken down much more rapidly than the ubiquitin **fusion** (**protein half-life** about 30 min). We show examples of the use of these reporters in the study of gene expression in Dictyostelium.

L6 ANSWER 23 OF 25 MEDLINE on STN DUPLICATE 12
ACCESSION NUMBER: 1999074273 MEDLINE
DOCUMENT NUMBER: PubMed ID: 9857028
TITLE: Generation of destabilized **green fluorescent protein** as a transcription reporter.
AUTHOR: Li X; Zhao X; Fang Y; Jiang X; Duong T; Fan C; Huang C C; Kain S R
CORPORATE SOURCE: Laboratories, Inc., Palo Alto, California 94303, USA.. xqli@clontech.com
SOURCE: Journal of biological chemistry, (1998 Dec 25) 273 (52) 34970-5.
Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199902
ENTRY DATE: Entered STN: 19990216
Last Updated on STN: 19990216
Entered Medline: 19990203

AB The **green fluorescent protein** (**GFP**) is a widely used reporter in gene expression and protein localization studies. **GFP** is a stable protein; this property allows its accumulation and easy detection in cells. However, this stability also limits its application in studies that require rapid reporter turnover. We created a destabilized **GFP** for use in such studies by fusing amino acids 422-461 of the degradation domain of mouse ornithine decarboxylase (MODC) to the C-terminal end of an enhanced variant of **GFP** (**EGFP**). The **fusion protein**, unlike **EGFP**, was unstable in the presence of cycloheximide and had a fluorescence **half-life** of 2 h. Western blot analysis indicated that the fluorescence decay of **EGFP**-MODC-(422-461) was correlated with degradation of the **fusion protein**. We mutated key amino acids in the PEST sequence of **EGFP**-MODC-(422-461) and identified several mutants with variable half-lives. The suitability of destabilized **EGFP** as a transcription reporter was tested by linking it to NFkappaB binding sequences and monitoring tumor necrosis factor alpha-mediated NFkappaB activation. We obtained time course induction and dose response kinetics similar to secreted alkaline phosphatase obtained in transfected cells. This result did not occur when unmodified **EGFP** was used as the reporter. Because of its autofluorescence, destabilized **EGFP** can be used to directly correlate gene induction with biochemical change, such as NFkappaB translocation to the nucleus.

L6 ANSWER 24 OF 25 MEDLINE on STN DUPLICATE 13
ACCESSION NUMBER: 1999039028 MEDLINE
DOCUMENT NUMBER: PubMed ID: 9821587
TITLE: Dual-function reporter protein for analysis of gene expression in living cells.
AUTHOR: Day R N; Kawecki M; Berry D
CORPORATE SOURCE: University of Virginia Health Sciences Center,

Charlottesville, USA.
SOURCE: BioTechniques, (1998 Nov) 25 (5) 848-50, 852-4, 856.
Journal code: 8306785. ISSN: 0736-6205.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199901
ENTRY DATE: Entered STN: 19990202
Last Updated on STN: 19990202
Entered Medline: 19990119

AB The firefly luciferase (Luc) protein and the jellyfish **green fluorescent protein** (**GFP**) are two commonly used molecular reporters that can be detected noninvasively in living cells. The properties that make **GFP** or Luc useful for a particular experimental application are quite distinct. A recombinant protein with both fluorescent and bioluminescent characteristics might take advantage of the strengths of both reporters. An expression vector encoding a chimeric protein in which **GFP** was tethered to Luc through a 19-amino acid linker was prepared and characterized. Western blotting with antibodies specific for either **GFP** or Luc showed that a protein of appropriate size was expressed in transfected cells. Fluorescence microscopy revealed bright green fluorescence from transfected cells, indicating proper formation of the **GFP** chromophore. Luc enzymatic activity in protein extracts from transfected cells showed that Luc was fully functional. The treatment of living cell cultures stably expressing the **GFP**-Luc **fusion protein** with the protein translation-inhibitor cycloheximide (Chx) was used to show that the **half-life** for Luc protein activity was approximately 2 h at 37 degrees C. The utility of this dual-function reporter protein was shown by the identification of single living cells expressing the chimeric protein within a population by fluorescence microscopy, followed by quantification of Luc activity from the same living cells.

L6 ANSWER 25 OF 25 CAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1998:775363 CAPLUS
DOCUMENT NUMBER: 130:92295
TITLE: Dual-function reporter protein for analysis of gene expression in living cells
AUTHOR(S): Day, Richard N.; Kawecki, Margaret; Berry, Diana
CORPORATE SOURCE: University of Virginia Health Sciences Center, Charlottesville, VA, USA
SOURCE: BioTechniques (1998), 25(5), 848, 850, 852-854, 856
CODEN: BTNQDO; ISSN: 0736-6205
PUBLISHER: Eaton Publishing Co.
DOCUMENT TYPE: Journal
LANGUAGE: English

AB The firefly luciferase (Luc) protein and the jellyfish **green fluorescent protein** (**GFP**) are two commonly used mol. reporters that can be detected noninvasively in living cells. The properties that make **GFP** or Luc useful for a particular exptl. application are quite distinct. A recombinant protein with both fluorescent and bioluminescent characteristics might take advantage of the strengths of both reporters. An expression vector encoding a chimeric protein in which **GFP** was tethered to Luc through a 19-amino acid linker was prepared and characterized. Western blotting with antibodies specific for either **GFP** or Luc showed that a protein of appropriate size was expressed in transfected cells. Fluorescence microscopy revealed bright green fluorescence from transfected cells, indicating proper formation of the **GFP** chromophore. Luc enzymic activity in protein exts. from transfected cells showed that Luc was fully functional. The treatment of living cell cultures stably expressing the **GFP**-Luc **fusion protein** with the protein

translation-inhibitor cycloheximide (Chx) was used to show that the **half-life** for Luc protein activity was approx. 2 h at 37°. The utility of this dual-function reporter protein was shown by the identification of single living cells expressing the chimeric protein within a population by fluorescence microscopy, followed by quantification of Luc activity from the same living cells.

REFERENCE COUNT: 28 THERE ARE 28 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

=> d his

(FILE 'HOME' ENTERED AT 16:42:26 ON 09 JUN 2004)

FILE 'MEDLINE, BIOTECHDS, EMBASE, BIOSIS, SCISEARCH, CANCERLIT, CAPLUS' ENTERED AT 16:42:36 ON 09 JUN 2004

L1 53940 S LI X?/AU OR KAIN S?/AU
L2 7357 S FUSION PROTEIN AND (FLUORESCENT PROTEIN)
L3 9189 S FUSION PROTEIN AND (ENHANC## GREEN FLUORESCENT PROTEIN OR GFP
L4 1417 S FUSION PROTEIN AND (ECFP OR EYFP OR EGFP)
L5 66 S (L2 OR L3 OR L4) AND HALF LIFE
L6 25 DUP REM L5 (41 DUPLICATES REMOVED)

=> s l1 and (l2 or l3 or l4)
L7 53 L1 AND (L2 OR L3 OR L4)

=> dup rem l7
PROCESSING COMPLETED FOR L7
L8 24 DUP REM L7 (29 DUPLICATES REMOVED)

=> d ibib abs l8 1-24

L8 ANSWER 1 OF 24 MEDLINE on STN DUPLICATE 1
ACCESSION NUMBER: 2004185149 MEDLINE
DOCUMENT NUMBER: PubMed ID: 15081407
TITLE: Genomic organization, expression, and subcellular localization of mouse mitochondrial seryl-tRNA synthetase.
AUTHOR: Gibbons William J Jr; Yan Qingfeng; Li Ronghua; **Li Xiaoming**; Guan Min-Xin
CORPORATE SOURCE: Division and Program in Human Genetics and Center for Hearing and Deafness Research, Cincinnati Children's Hospital Medical Center, Cincinnati, OH 45229, USA.
CONTRACT NUMBER: DC04958 (NIDCD)
DC05230 (NIDCD)
NS44015 (NINDS)
SOURCE: Biochemical and biophysical research communications, (2004 May 7) 317 (3) 774-8.
Journal code: 0372516. ISSN: 0006-291X.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200405
ENTRY DATE: Entered STN: 20040415
Last Updated on STN: 20040520
Entered Medline: 20040519

AB We report here the identification and characterization of the mouse mitochondrial seryl-tRNA synthetase (mtSerRS). The genomic organization of mouse mtSerRS has been elucidated. The mouse mtSerRS gene containing 16 exons encodes a 519 residue protein with a strong homology to the mitochondria-like seryl-tRNA synthetase of bacteria, yeast, and other homologs. The mouse mtSerRS is ubiquitously expressed in various tissues, but more abundantly in tissues with high metabolic rates including heart and liver. Surprisingly, this gene, unlike other nuclear genes encoding

mitochondrial proteins, exhibited a low expression in skeletal muscle and brain. Furthermore, immunofluorescence analysis of NIH3T3 cells expressing the mtSerRS-**GFP fusion protein** demonstrated that the mouse mtSerRS localizes in mitochondrion. These observations suggest that the mouse mtSerRS is an evolutionarily conserved protein involved in aminoacylation. Thus, it may play a role in the fidelity in mitochondrial translation and pathogenesis of deafness-associated mutations in the mitochondrial tRNA(Ser(UCN)).

L8 ANSWER 2 OF 24 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
ACCESSION NUMBER: 2003-21811 BIOTECHDS
TITLE: Monitoring tumor treatment in a subject, e.g., mouse, harboring a solid tumor comprises treating the subject with bacteria that express a first **fluorescent protein** of a first color; retro virus vector or Salmonella sp.-expressing **fluorescent protein** gene transfer and expression in tumor animal model for use in cancer gene therapy
AUTHOR: ZHAO M M D P D; **LI X M D P D**; YANG M M D P D; XU M M D; JIANG P M D; LI L M D
PATENT ASSIGNEE: ANTICANCER INC
PATENT INFO: WO 2003057007 17 Jul 2003
APPLICATION INFO: WO 2002-US41822 31 Dec 2002
PRIORITY INFO: US 2001-345699 31 Dec 2001; US 2001-345699 31 Dec 2001
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2003-587063 [55]
AN 2003-21811 BIOTECHDS
AB DERWENT ABSTRACT:
NOVELTY - Monitoring (M1) the process of tumor treatment in a subject comprising observing the presence, absence or intensity of fluorescence in the solid tumor, where the subject has been treated with bacteria that express a first **fluorescent protein** of a first color, where dispersement over time of the first **fluorescent protein** in the tumor indicates the progress of treatment, is new.

USE - M1 is used for monitoring tumor treatment in a subject, e.g., mouse, rat or rabbit that has been modified to contain tumor cells that express the second **fluorescent protein** or for monitoring tumor treatment in a human that has been administered a viral vector, e.g. retroviral vector, for expression of the second **fluorescent protein** (claimed).

ADVANTAGE - M1 permits visualization of the progress of treatment in live subjects so that treatments can be modified according to their efficacy.

EXAMPLE - **Green-fluorescent protein** (**GFP**) labeled Salmonella were injected into a red-**fluorescent protein** (RFP)-labeled U-87 human glioma in a nude mouse. A phosphate buffered saline (PBS) solution (10 microliter) containing 1 x 10 to the power of 8 **GFP**-labeled Salmonella was injected into the RFP-labeled U-87 human glioma. The **GFP** -labeled Salmonella was imaged in the glioma immediately after injection and one day after injection. The **GFP** could be seen against the background of RFP and had spread after one day.(17 pages)

L8 ANSWER 3 OF 24 CAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 2003:656310 CAPLUS
DOCUMENT NUMBER: 139:174827
TITLE: Methods and kits for isolating and characterizing short-lived proteins and arrays derived therefrom for use in drug screening
INVENTOR(S): **Li, Xianqiang**; Jiang, Xin
PATENT ASSIGNEE(S): USA
SOURCE: U.S. Pat. Appl. Publ., 30 pp., Cont.-in-part of U.S.

Ser. No. 53,230.
CODEN: USXXCO
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 3
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 2003157540	A1	20030821	US 2003-347160	20030116
US 2003134287	A1	20030717	US 2002-53230	20020116
US 2003134288	A1	20030717	US 2002-53516	20020116

PRIORITY APPLN. INFO.: US 2002-53230 A2 20020116
US 2002-53516 A2 20020116

AB Compns., kits and methods are provided for isolating and characterizing short-lived proteins. The method comprises taking a library of cells wherein each cell in the library expresses a **fusion protein** comprising a reporter protein and a protein encoded by a sequence from a cDNA library derived from a sample of cells.. The sequence from the cDNA library is varied within the cell library and the rate of protein expression or degradation by cells in the library is modified. A population of cells is selected from the library of cells based on the population of cells having different reporter signal intensities than other cells in the library wherein the difference between intensities is indicative of the population of cells expressing shorter lived fusion proteins than the fusion proteins expressed by the other cells in the library and determining protein sequences of the fusion proteins of the selected population of cells. Also provided are oligonucleotide, protein and antibody arrays derived from short-lived proteins. The arrays can be used for efficiently profiling expression of short-lived proteins, screening for binding agents and comparing expression levels under different conditions.

L8 ANSWER 4 OF 24 MEDLINE on STN DUPLICATE 2
ACCESSION NUMBER: 2003598995 MEDLINE
DOCUMENT NUMBER: PubMed ID: 14680828
TITLE: Identification and characterization of mouse GTPBP3 gene encoding a mitochondrial GTP-binding protein involved in tRNA modification.
AUTHOR: **Li Xiaoming**; Guan Min-Xin
CORPORATE SOURCE: Division and Program in Human Genetics and Center for Hearing and Deafness Research, Cincinnati Children's Hospital Medical Center, Cincinnati, OH 45229, USA.
CONTRACT NUMBER: DC04958 (NIDCD)
DC05230 (NIDCD)
NS44015 (NINDS)
SOURCE: Biochemical and biophysical research communications, (2003 Dec 19) 312 (3) 747-54.
Journal code: 0372516. ISSN: 0006-291X.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200403
ENTRY DATE: Entered STN: 20031219
Last Updated on STN: 20040313
Entered Medline: 20040312

AB We report here the identification and characterization of mouse GTPBP3 encoding a mitochondrial GTPase. A full-length GTPBP3 cDNA has been isolated and the genomic organization of GTPBP3 has been elucidated. The mouse GTPBP3 gene containing 9 exons encodes a 486 residue protein with a strong homology to the GTPBP3-like proteins of bacteria, yeast, and other homologs, related to tRNA modification. The mouse GTPBP3 is ubiquitously

expressed in various tissues, but abundantly in tissues with high metabolic rates including heart, liver, and brain. Surprisingly, this gene, unlike its human homolog, exhibited a low expression in skeletal muscle. Furthermore, immunofluorescence analysis of NIH3T3 cells expressing GTPBP3-**GFP fusion protein** demonstrated that the mouse Gtpbp3 localizes in mitochondrion. These observations suggest that the mouse Gtpbp3 is an evolutionarily conserved mitochondrial GTP-binding protein involved in the tRNA modification. Thus, it may modulate the translational efficiency and accuracy of codon-anticodon base pairings on the decoding region of mitochondrial ribosomes.

L8 ANSWER 5 OF 24 MEDLINE on STN DUPLICATE 3
ACCESSION NUMBER: 2004035245 MEDLINE
DOCUMENT NUMBER: PubMed ID: 14735063
TITLE: Does lens intrinsic membrane protein MP19 contain a membrane-targeting signal?.
AUTHOR: Chen Tong; **Li XiaLian**; Yang Yu; Erdene Agiimaa Gan; Church Robert L
CORPORATE SOURCE: Department of Ophthalmology, Emory University School of Medicine, Atlanta, GA, USA.
CONTRACT NUMBER: C06 EY06307 (NEI)
P30 EY06360 (NEI)
R01 EY11516 (NEI)
R01 EY12301 (NEI)
T32 EY07092 (NEI)
SOURCE: Molecular vision [electronic resource], (2003 Dec 22) 9 735-46.
Journal code: 9605351. ISSN: 1090-0535.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200401
ENTRY DATE: Entered STN: 20040122
Last Updated on STN: 20040128
Entered Medline: 20040127

AB PURPOSE: Lens intrinsic membrane protein MP19 is the second most abundant major protein of the lens fiber cell membrane and appears to be specific to the lens. Different mutations of this protein are known to cause cataract in both humans and mice. To date, the function of MP19 in the lens is not known, nor is the mechanism by which the protein migrates to the cell membrane. The goal of this study was to determine whether or not MP19 distributes to the cell membrane directed by a peptide signal within the sequence of the molecule. METHODS: Using PCR, MP19 cDNA was truncated to yield separate fragments coding for the first 25, 36, and 64 amino acids of the MP19 polypeptide chain. These PCR fragments were further cloned into mammalian expression vector pcDNA4/TO, a tetracycline-regulated vector that, upon induction with tetracycline, allows expression of cDNA inserts within the vector. These vectors expressed each of the MP19 truncated fragments fused to **EGFP**. Each of the prepared plasmids was transfected into T-REx-293 cells using FuGene 6. Cloned cell lines from each of these transfections were obtained and used in the studies. The fluorescent expressed protein was viewed using confocal microscopy. Proteins from the different cell lines were isolated by different membrane extraction methods and western blot analysis was carried out to further determine the localization of expressed MP19 and MP19 truncated fragments. RESULTS: Cell lines expressing intact MP19/**EGFP** (with **EGFP** fused to the COOH-terminal end of MP19, MP19G) **fusion protein** were observed to traffic MP19 to the cell membrane, where it appeared to sequester in rather large pools. All of the MP19 truncations (with **EGFP** fused to the COOH-terminal end of each truncation; MP19-25G, MP19-36G, and MP19-64G) appeared to also traffic **EGFP** to the cell membrane. MP19-25G

and MP19-36G did not distribute uniformly on the membrane, but appeared to localize into smaller, punctate "spots" of fluorescent material. MP19-64G distributed on the membrane similarly to MP19-25G and MP19-36G, however, the punctate areas of fluorescent material were considerably larger and similar to that demonstrated by intact MP19G. Western blot analysis of isolated total membranes, intrinsic membranes, and lipid rafts showed that MP19G and MP19-64G were associated with the intrinsic membrane fraction while MP19-25G and MP19-36G were at least 75% associated with the intrinsic membrane fraction. All of the preparations appeared to be at least 50% associated with membrane lipid rafts. However, when **EGFP**/MP19-25 and **EGFP**/MP19-36 (with **EGFP** fused to the NH2-terminal end of the truncated peptide, GMP19-25 or GMP19-36) were expressed, the **fusion protein** was observed to remain completely soluble in the cytoplasm, identical to expressed **EGFP** alone. Western blots of these two fusion proteins also indicated that the product did not associate with the cell membrane. In contrast, when **EGFP**/MP19 (with **EGFP** fused to the NH2-terminal end of intact MP19, GMP19) was expressed, the **fusion protein** did integrate into the cell membrane, identical to MP19G. Western blot analysis revealed that GMP19 also associated with lipid rafts, identical to intact MP19G. CONCLUSIONS: It appears that the first 25 amino acids of the MP19 molecule are sufficient to target the protein to the cell membrane, and apparently integrate into the membrane. With the addition of more amino acids, the polypeptide distributes in the membrane similarly to that of the intact MP19 molecule. It appears that the first 25 amino acids of the MP19 molecule is, indeed, a membrane signal and integration sequence. Also, at least part of these 25 amino acids must integrate into the cell membrane, but not extend through the cell membrane.

L8 ANSWER 6 OF 24 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 2004:91958 BIOSIS
DOCUMENT NUMBER: PREV200400085150
TITLE: Sop2p, a homolog of the Arp2/3 complex p41 protein, is a novel hsp27 binding protein.
AUTHOR(S): **Li, Xiangquan** [Reprint Author]; Ransom, Richard F. [Reprint Author]; Jia, Yifeng; Welsh, Michael J.; Smoyer, William E. [Reprint Author]
CORPORATE SOURCE: Pediatric Nephrology, University of Michigan, Ann Arbor, MI, USA
SOURCE: Journal of the American Society of Nephrology, (November 2003) Vol. 14, No. Abstracts Issue, pp. 2A. print.
Meeting Info.: Meeting of the American Society of Nephrology Renal Week. San Diego, CA, USA. November 12-17, 2003. American Society of Nephrology.
CODEN: JASNEU. ISSN: 1046-6673.
DOCUMENT TYPE: Conference; (Meeting)
Conference; (Meeting Poster)
Conference; Abstract; (Meeting Abstract)
LANGUAGE: English
ENTRY DATE: Entered STN: 11 Feb 2004
Last Updated on STN: 11 Feb 2004

L8 ANSWER 7 OF 24 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 2003:554478 BIOSIS
DOCUMENT NUMBER: PREV200300551752
TITLE: DOES LENS INTRINSIC MEMBRANE PROTEIN MP19 CONTAIN A MEMBRANE TRANSPORT AND INSERTION SIGNAL?.
AUTHOR(S): Chen, T. [Reprint Author]; **Li, X.** [Reprint Author]; Yang, Y. [Reprint Author]; Church, R. L. [Reprint Author]
CORPORATE SOURCE: Ophthalmology Eye Center, Emory University, Atlanta, GA, USA
SOURCE: ARVO Annual Meeting Abstract Search and Program Planner,

(2003) Vol. 2003, pp. Abstract No. 4482. cd-rom.
Meeting Info.: Annual Meeting of the Association for Research in Vision and Ophthalmology. Fort Lauderdale, FL, USA. May 04-08, 2003. Association for Research in Vision and Ophthalmology.
DOCUMENT TYPE: Conference; (Meeting)
Conference; Abstract; (Meeting Abstract)
LANGUAGE: English
ENTRY DATE: Entered STN: 26 Nov 2003
Last Updated on STN: 26 Nov 2003

AB Purpose: Lens intrinsic membrane protein MP19 is the second most abundant major protein of the lens fiber cell membrane. MP19 transports to the cell membrane through the classic secretory protein pathway. The goal of this study was to determine whether or not MP19 distributes to the cell membrane directed by a signal. Methods: Using PCR, MP19 cDNA was truncated to yield separate fragments coding for the first 25, 36, and 64 amino acids of the MP19 polypeptide chain. These PCR fragments were further cloned into mammalian expression vector pcDNA4/TO, a tetracycline-regulated vector which, upon induction with tetracycline allows expression of cDNA inserts within the vector. These truncated cDNA fragments were separately cloned into the vector so that the fragments were at the 5'-end of an **EGFP** coding cDNA. These vectors expressed each of the MP19 truncated fragments fused to **EGFP**. Each of the prepared plasmids was transfected into TRx-293 cells using FuGene 6. Cloned cell lines from each of these transfections were obtained and used in the studies. The fluorescent expressed protein was viewed using confocal microscopy. Proteins from the different cell lines were isolated by different membrane extraction methods and western blot analysis was carried out to further determine the localization of expressed MP19 and MP19 truncated fragments. Results: Cell lines expressing intact MP19/**EGFP fusion protein** were observed to traffic MP19 to the cell membrane, where it appeared to sequester in rather large pools or areas. All of the MP19 truncations appeared to traffic **EGFP** to the cell membrane also. MP19-25 and MP19-36 did not distribute uniformly on the membrane, but appeared to localize into very small, punctate "spots" of fluorescent material. MP19-64 distributed on the membrane very similar to MP19-25 and MP19-36, however, the punctate "spots" of flunrescent material were considerably larger. Western blot analysis of isolated total membranes, intrinsic membranes, and lipid rafts showed that MP19 and MP19-64 were associated with the intrinsic membrane fraction while MP19-25 and -36 were at least 75% associated with the intrinsic membrane fraction. All of the preparations appeared to be about 50% associated with membrane lipid rafts. Conclusions: It appears that the first 25 amino acids of the MP19 molecule are sufficient to target the protein to the cell membrane, and apparently insert into the membrane. With the addition of more amino acids, the polypeptide distributes in the membrane very similar to that of the intact MP19 molecule. It appears that the first 25 amino acids of the MP19 molecule is, indeed, a membrane signal and insertion sequence.

L8 ANSWER 8 OF 24 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN DUPLICATE 4
ACCESSION NUMBER: 2002:914972 SCISEARCH
THE GENUINE ARTICLE: 610JF
TITLE: Preliminary study of LRRC4 protein: Bioinformatic analysis, fusion expression in eukaryote and prokaryote
AUTHOR: Wang J R (Reprint); Dong L; Jiang M; Tan C; **Li X L**; Xiang J J; Fan S Q; Peng C; Tang K; Li G Y
CORPORATE SOURCE: Cent S Univ, Xiangya Sch Med, Changsha 410078, Peoples R China; People Hosp Shenzhen Lantian District, Shenzhen 518081, Peoples R China
COUNTRY OF AUTHOR: Peoples R China
SOURCE: PROGRESS IN BIOCHEMISTRY AND BIOPHYSICS, (OCT 2002) Vol. 29, No. 5, pp. 696-701.
Publisher: SCIENCE CHINA PRESS, 16 DONGHUANGCHENGGEN NORTH

ST, BEIJING 100717, PEOPLES R CHINA.
ISSN: 1000-3282.
DOCUMENT TYPE: Article; Journal
LANGUAGE: Chinese
REFERENCE COUNT: 14
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB In previous study, a novel gene, LRRC4, a member of leucine-rich repeat (LRR) superfamily was cloned. Expression analysis indicated that LRR may play an Important rote in the central nervous system. To investigate the function and the structure-function relationship of LRRC4, full length coding region was amplified and subcloned into pGEM T Easy vector. Further, the recombinant plasmid, pEGFP-C1 /LRRC4, was constructed and transfected transiently into U251 cell. Under the fluorescence microscope, the green fluorescence produced by LRRC4 **fusion protein** was observed on the cytoplasmic membrane. Consistent to prediction by bioinformatics, this result indicated that product of LRRC4 is a membrane protein. In addition, the recombinant of LRRC4, pGEX-4T-2/LRRC4 and truncated LRRC4 recombinant, pGEX-4T-2/mLRRC4, were constructed and transformed into E. coli BL21. Induced by 0.5 mmol/L IPTG, The band corresponding to **fusion protein** were observed in SDS-PAGE as expected. Together with bioinformatic analysis of LRRC4 protein, these results establish the basis for functional study of LRRC4.

L8 ANSWER 9 OF 24 MEDLINE on STN DUPLICATE 5
ACCESSION NUMBER: 2002622351 MEDLINE
DOCUMENT NUMBER: PubMed ID: 12379840
TITLE: Localization of lens intrinsic membrane protein MP19 and mutant protein MP19(To3) using fluorescent expression vectors.
AUTHOR: Chen Tong; **Li XiaLian**; Yang Yu; Church Robert L
CORPORATE SOURCE: Department of Ophthalmology, Emory University School of Medicine, Atlanta, GA, USA.
CONTRACT NUMBER: C06 EY06307 (NEI)
P30 EY06360 (NEI)
R01 EY11516 (NEI)
R01 EY12301 (NEI)
T32 EY07092 (NEI)
SOURCE: Molecular vision [electronic resource], (2002 Oct 11) 8 372-88.
Journal code: 9605351. ISSN: 1090-0535.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200211
ENTRY DATE: Entered STN: 20021017
Last Updated on STN: 20021213
Entered Medline: 20021104

AB PURRPOSE: MP19 is the second most abundant major intrinsic protein of the lens fiber cell membrane. A specific heritable mutation at amino acid 15 in the MP19 protein, termed MP19To3, results in total cataract and microphthalmia in the mouse. The goals of this study were to determine the specific localization of MP19 in the cell membrane and to determine whether the mutant MP19To3 protein migrates to the cell membrane in a similar fashion to normal MP19. METHODS: MP19 and MP19To3 cDNAs were cloned into two different sets of expression vectors. The first set was composed of two vectors, pEGFP-N1 and pDsRed2-N1. The first vector expressed **green fluorescent protein** and the second expressed a red **fluorescent protein** when transfected into mammalian cells. The two lens membrane protein cDNAs were separately cloned into the vectors so that the cDNA was at the 5'-end of the **fluorescent protein** coding DNA. These vectors expressed each of the lens proteins fused to the **fluorescent protein** upon transfection into mammalian cell cultures. The

second vector set was a single vector, pcDNA4/TO which must be induced in the transfected cells by tetracycline in order to express the cloned cDNAs. Each of the membrane cDNAs coupled to the **fluorescent protein** coding region was cut out of the first vector set and cloned into pcDNA4/TO and stable clones were isolated. Each of the prepared plasmids was transfected into human and chick embryo lens epithelial cells and human T-RexTM-293 cells. The fluorescent cells were viewed using confocal and episcopic-fluorescence microscopy. RESULTS: Each of the transfected plasmids expressed **fluorescent protein** in all three cell lines. MP19 was observed to transport to the cell membrane. When compared to the distribution of another, separate **fusion protein** consisting of a signal peptide that targets to cell membranes fused to **EGFP**, MP19 did not distribute uniformly on the membrane, but appeared to localize into "spots" or pools of fluorescent material around the cell membrane. In contrast, MP19To3 protein appeared to not distribute to the cell membrane; it instead appeared to collect in a particular subcellular compartment within the cell. CONCLUSIONS: The distribution of MP19 and MP19To3 in the cell appeared to be quite distinct. MP19 was observed to distribute to the cell membrane while MP19To3 did not. The fact that the MP19To3 did not traffic to the membrane, instead appearing to be trapped within a subcellular compartment within the cell sheds further light on the cause of the cataract and microphthalmia observed in the MP19To3 mutation, and further sheds information on the pathway of MP19 transport to the cell membrane.

L8 ANSWER 10 OF 24 CAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 2003:205871 CAPLUS
DOCUMENT NUMBER: 139:20265
TITLE: Expression and location analysis of NAG7 gene associated with nasopharyngeal carcinoma
AUTHOR(S): Tan, Chen; Dong, Li; Li, Jiang; Cao, Li; Peng, Cong; **Li, Xiaoling**; Li, Guiyuan
CORPORATE SOURCE: Xiangya School of Medicine, Central South University, Changsha, Hunan Province, 410078, Peop. Rep. China
SOURCE: Shengming Kexue Yanjiu (2002), 6(1), 74-78
CODEN: SKYAFL; ISSN: 1007-7847
PUBLISHER: Shengming Kexue Yanjiu Bianji Weiyuanhui
DOCUMENT TYPE: Journal
LANGUAGE: Chinese
AB The expression of the NAG7 gene and location of the NAG7 coding protein were analyzed, as well as the relationship between the expression pattern of the protein and cell carcinogenesis. The NAG7 cDNA was amplified by PCR, NAG7 gene was prepared and an enhanced green fluorescence protein (**EGFP**) NAG7 gene was prepared The recombinant plasmid was transfected into HNE1 and COS-7 cells, and then the expression of NAG7 coding protein was observed by fluorescence microscopy. The results showed that the expressed **GFP**-NAG7 **fusion protein** generated striking green fluorescence in the cytoplasm in HNE1 and COS-7 cells. Green fluorescence was generated in most of COS-7 cells but in only a few HNE1 cells. This suggests that the differential expression in HNE1 might indicate involvement of NAG7 in nasopharyngeal carcinoma (NPC).

L8 ANSWER 11 OF 24 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 2003:165720 BIOSIS
DOCUMENT NUMBER: PREV200300165720
TITLE: Localization of Lens Intrinsic Membrane Proteins MP19, Cx50, and Mutant MP19To3 Using Fluorescent Expression Vectors.
AUTHOR(S): Chen, T. [Reprint Author]; **Li, X.** [Reprint Author]; Li, Z. [Reprint Author]; Yang, Y. [Reprint Author]; Church, R. L. [Reprint Author]
CORPORATE SOURCE: Ophthalmology, Emory University, Atlanta, GA, USA
SOURCE: ARVO Annual Meeting Abstract Search and Program Planner,

(2002) Vol. 2002, pp. Abstract No. 4637. cd-rom.
Meeting Info.: Annual Meeting of the Association For Research in Vision and Ophthalmology. Fort Lauderdale, Florida, USA. May 05-10, 2002.

DOCUMENT TYPE: Conference; (Meeting)
Conference; Abstract; (Meeting Abstract)

LANGUAGE: English

ENTRY DATE: Entered STN: 2 Apr 2003
Last Updated on STN: 2 Apr 2003

AB Purpose: MP19 and connexin50 (Cx50) are known to be intrinsic proteins of the lens fiber cell membrane. A specific mutation of MP19, termed MP19To3 leads to heritable cataract. The goals of this study were to determine the specific localization of MP19 and Cx50 in the cell membrane, and to determine whether the MP19To3 protein migrates to the cell membrane in a similar fashion to normal MP19. Methods: MP19, Cx50, and MP19To3 cDNAs were cloned into two different types of expression vectors. The first vector set was composed of two vectors, pEGFP-N1 and pDSRed2-N1. The first vector expressed **green fluorescent protein** and the second expressed a red **fluorescent protein** when transfected into mammalian cells. The three lens membrane protein cDNAs were separately cloned into the vectors so that the cDNA was at the 5'- end of the **fluorescent protein** coding DNA. These vectors expressed each of the lens proteins fused to the **fluorescent protein**. The second vector set was a single vector, pcDNA4/TO which must be induced in the transfected cells by tetracycline in order to express the cloned cDNAs. Each of the membrane cDNAs coupled to the **fluorescent protein** coding region was cut out of the first vector set and cloned into pcDNA4/TO and stable clones were isolated. Each of the prepared plasmids were transfected into mouse TRx-293 and human lens epithelial cells using FuGene 6. The fluorescent cells were viewed using confocal microscopy. Results: Each of the transfected plasmids expressed **fluorescent protein** in both cell lines. MP19 and Cx50 were observed to transport to the cell membrane. When compared to the distribution of a separate **fusion protein** consisting of a signal peptide that targets to cellular membranes fused to **EGFP**, both MP19 and Cx50 did not distribute uniformly on the membrane, but appeared to localize into "spots" or pools of fluorescent material around the cell membrane. Little co-localization of MP19 and Cx50 was observed. In contrast, MP19To3 protein appeared to not distribute to the cell membrane, it instead appeared to collect in vesicles within the cell. Conclusion: The distribution of MP19 and Cx50 around the cell membrane appeared to be distinct. Little co-localization of the two proteins was observed, indicating the possibly that the two lens membrane proteins function completely different from one another. The fact that the mutant MP19To3 did not traffic to the membrane, instead appearing to be trapped in vesicles within the cell may shed light on the cause of the cataract and microphthalmia observed in the To3 mutant animal.

L8 ANSWER 12 OF 24 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN

ACCESSION NUMBER: 2002-03910 BIOTECHDS

TITLE: Assaying the regulatory function of a transcriptional control sequence, for drug screening or for analyzing protein degradation, comprises employing rapidly degrading **Green Fluorescent Protein**-fusion proteins;
vector-mediated expression in host cell useful for gene expression profiling and transcription regulation analysis

AUTHOR: **Kain S; Li X**

PATENT ASSIGNEE: Clontech-Lab.

LOCATION: Palo Alto, CA, USA.

PATENT INFO: US 6306600 23 Oct 2001

APPLICATION INFO: US 1999-364946 30 Jul 1999

PRIORITY INFO: US 1999-364946 30 Jul 1999

DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2002-040198 [05]
AN 2002-03910 BIOTECHDS
AB Assaying the regulatory function of a transcriptional or translational control sequence, involves transfecting cells with an expression vector having a DNA sequence that codes for a fluorescent **fusion protein** comprising a **green fluorescent protein** (**GFP**) and a PEST (Pro Glu Ser Thr) sequence from the degradation domain of mouse ornithine-decarboxylase, is new. Also claimed are: assaying a test compound on the regulatory function of a transcriptional or translational control sequence involving: treating the transfected cell with the test compound that has no effect on the expression of the fluorescent **fusion protein**; and detecting an amount of fluorescence in the cells, where an increase or decrease in fluorescence in cells treated with the test compound compared to cells treated with the control compound indicates a positive or negative effect, respectively. Also disclosed is a rapidly-degrading genetically engineered fusion **fluorescent protein**. The method is useful for analyzing transcriptional regulation and/or cis-acting regulatory elements, studying protein degradation, studying processes involving multiple gene expression profiling, as well as drug screening. (25pp)

L8 ANSWER 13 OF 24 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
ACCESSION NUMBER: 2002-01055 BIOTECHDS
TITLE: New mammalian taste-cell-specific G-protein coupled receptor polypeptides for identifying compounds that modulate taste signaling are useful in food, to modulate the sweet taste of foods or drugs;
bacterium plasmid SAV115, plasmid SAV118, phage, virus, retro virus, linear, circular DNA vector-mediated **green fluorescent protein** gene transfer and expression in host cell
AUTHOR: Adler J E; Zozulya S; O'Connell S M; **Li X**; Staszewski L
PATENT ASSIGNEE: Senomyx
LOCATION: La Jolla, CA, USA.
PATENT INFO: WO 2001066563 13 Sep 2001
APPLICATION INFO: WO 2001-US7265 7 Mar 2001
PRIORITY INFO: US 2001-259227 3 Jan 2001; US 2000-187546 7 Mar 2000
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2001-582267 [65]
AN 2002-01055 BIOTECHDS
AB An isolated mouse, human or rat G-protein coupled receptor (I) is claimed. Also claimed are: a fragment of (I) containing 5-7 amino acids; a chimeric or **fusion protein** containing (I) and **green fluorescent protein**; a nucleic acid (II) encoding (I); a RNA molecule transcribed from (II); a mammal, bacterial plasmid, phage, virus, retro virus and linear or circular DNA containing (II); a host cell transfected with the above vectors that expresses (I) on its surface; a DNA array containing (II); a genomic DNA amplified by polymerase chain reaction with DNA primers; plasmid SAV115 and plasmid SAV118 encoding mouse (I) and rat (I), respectively; a protein array containing (I); and an antibody that binds to (I). Also disclosed as new are: agonist and antagonist of (I); and kits for drug screening modulators of (I) family members. Fragments of (I) can be used for drug screening for a compound that activates and/or modulates taste-signaling. (119pp)

L8 ANSWER 14 OF 24 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
ACCESSION NUMBER: 2001-01398 BIOTECHDS
TITLE: New rapidly degrading **green fluorescent**

protein (**GFP**)-fusion proteins, useful in a variety of research applications, e.g. as a reporter in determining gene expression and protein localization; constructed by of fusing C-terminus amino acids of the degradation domain mouse ornithine-decarboxylase

AUTHOR: **Li X; Kain S**
PATENT ASSIGNEE: Clontech-Lab.
LOCATION: Palo Alto, CA, USA.
PATENT INFO: US 6130313 10 Oct 2000
APPLICATION INFO: US 1998-62102 17 Apr 1998
PRIORITY INFO: US 1998-62102 17 Apr 1998
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2000-655680 [63]
AN 2001-01398 BIOTECHDS
AB A **fusion protein** comprising **green fluorescent protein** (**GFP**) and a PEST sequence is claimed. The protein has a half-life of no more than ten hours. The **fusion protein** is useful in research applications, e.g. as a genetic reporter for analyzing transcriptional regulation and/or cis-acting regulatory element, as a tool for identifying and dissecting degradation domains of short-lived proteins, or in drug screening assays. The method provides a rapid turnover or destabilized **GFP** that can be used in research applications where prior method GFPs cannot. A rapid turnover **GFP** permits development of a stable cell line, which expresses the **GFP** gene since toxic levels of **GFP** are avoided, as the **GFP** protein is quickly degraded. Also disclosed are: an isolated DNA molecule encoding a **GFP** **fusion protein** with a half life decreased from that of wild type **GFP**; a vector capable of expressing the isolated DNA molecule; a method of producing a stable cell line that expresses **GFP** by transfecting cells with the vector; and a method of labelling cells with a transient **GFP** reporter. (18pp)

L8 ANSWER 15 OF 24 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 2001:163409 BIOSIS
DOCUMENT NUMBER: PREV200100163409
TITLE: IkappaBEGFP constructs, cell lines and methods of use.
AUTHOR(S): **Li, Xianqiang** [Inventor]
CORPORATE SOURCE: ASSIGNEE: Clontech Laboratories, Inc.
PATENT INFORMATION: US 6093808 July 25, 2000
SOURCE: Official Gazette of the United States Patent and Trademark Office Patents, (July 25, 2000) Vol. 1236, No. 4. e-file. CODEN: OGUPE7. ISSN: 0098-1133.
DOCUMENT TYPE: Patent
LANGUAGE: English
ENTRY DATE: Entered STN: 4 Apr 2001
Last Updated on STN: 15 Feb 2002
AB The present invention provides **fusion protein** comprising IkappaB and **green fluorescent protein**. Also provided is DNA encoding this protein and vector expressing such DNA and various methods of using a **fusion protein** comprising IkappaB and **green fluorescent protein**.

L8 ANSWER 16 OF 24 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 2000:408537 BIOSIS
DOCUMENT NUMBER: PREV200000408537
TITLE: IkappaBEGFP constructs, cell lines and methods of use.
AUTHOR(S): **Li, Xianqiang** [Inventor, Reprint author]
CORPORATE SOURCE: Palo Alto, CA, USA
ASSIGNEE: Clontech Laboratories, Inc., Palo Alto, CA, USA
PATENT INFORMATION: US 6037133 March 14, 2000
SOURCE: Official Gazette of the United States Patent and Trademark

Office Patents, (Mar. 14, 2000) Vol. 1232, No. 2. e-file.
CODEN: OGUPE7. ISSN: 0098-1133.
DOCUMENT TYPE: Patent
LANGUAGE: English
ENTRY DATE: Entered STN: 27 Sep 2000
Last Updated on STN: 8 Jan 2002

AB The present invention provides **fusion protein** comprising IkappaB and **green fluorescent protein**. Also provided is DNA encoding this protein and vector expressing such DNA and various methods of using a **fusion protein** comprising IkappaB and **green fluorescent protein**.

L8 ANSWER 17 OF 24 CAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 2000:401962 CAPLUS
DOCUMENT NUMBER: 133:39141
TITLE: Cis-element reporter constructs for measuring transcription and their applications
INVENTOR(S): **Li, Xianqiang**; Zhao, Xiaoning; Jiang, Xin; Fang, Yu; Duong, Tommy; **Kain, Steve**
PATENT ASSIGNEE(S): Clontech Laboratories, Inc., USA
SOURCE: PCT Int. Appl., 42 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2000034435	A2	20000615	WO 1999-US28451	19991201
WO 2000034435	A3	20001012		

W: JP
RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE
PRIORITY APPLN. INFO.: US 1998-206887 A 19981208

AB The present invention provides cis element-reporter constructs for measuring transcription. These constructs contain one of a series of selected cis elements, a promoter and a reporter gene controlled by these transcription regulatory elements. These cis-elements include NF-κB binding sequence , HRE (heat responsive element), Myc binding sequence, p53 binding sequence, NFAT (nuclear factor of activated T-cell) binding sequence , AP1(activator protein 1) element, SRE (serum responsive element), GRE (glucocorticoid responsive element), CRE3 (cAMP responsive element 3) and CRE5. Examples of reporter genes are secreted alkaline phosphatase (SEAP), destabilized **green fluorescent protein** (d2EGFP, a **fusion protein** of the enhanced **GFP** variant (**EGFP**) containing PEST domain from mouse ornithine decarboxylase) and luciferase. The promoter in the vectors can be selected from thymidine kinase gene, or gonadotropin α gene. Studies of tumor necrosis factor (TNF)-mediated or serum-mediated transcription induction in HEK 293 cells are presented. The assay can be used for establishing a functional status profiles of transcription factors, examining and quickly identifying biol. markers for human diseases, and drug validation.

L8 ANSWER 18 OF 24 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
ACCESSION NUMBER: 2000-01528 BIOTECHDS
TITLE: New **fusion protein** useful for studying cell localization, for studying cell lineage and for assaying activation or deactivation of transcriptional or translational elements;
green fluorescent protein and mouse ornithine-decarboxylase **fusion**

protein expression in host cell
AUTHOR: **Li X; Kain S**
PATENT ASSIGNEE: Clontech-Lab.
LOCATION: Palo Alto, CA, USA.
PATENT INFO: WO 9954348 28 Oct 1999
APPLICATION INFO: WO 1998-US24323 13 Nov 1998
PRIORITY INFO: US 1998-62102 17 Apr 1998
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2000-013225 [01]
AN 2000-01528 BIOTECHDS
AB A **fusion protein** containing a **fluorescent protein** related to **green fluorescent protein** and mouse ornithine-decraboxylase (EC-4.1.1.17) degradation domain C-terminal amino acids and having a half-life of no more than 10 hr, is new. Also claimed are: a DNA molecule encoding the **fusion protein**; a vector; a stable cell line that expresses a **fluorescent protein**; and production of the cell line. The protein can be used for assaying activation or deactivation of transcriptional or translational elements with a transient fluorescent reporter protein. The protein can be used to study cell lineage. It can also be linked with different enhancer elements and used to monitor biological processes e.g. heat response, response to cyclic-AMP etc. (49pp)

L8 ANSWER 19 OF 24 CAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1999:691296 CAPLUS
DOCUMENT NUMBER: 131:296215
TITLE: IκB- **enhanced green fluorescent protein** (IκB- **EGFP**) **fusion protein**, DNA molecule encoding it and its use in identifying compounds that activate NFκB
INVENTOR(S): **Li, Xianqiang**
PATENT ASSIGNEE(S): Clontech Laboratories, Inc., USA
SOURCE: PCT Int. Appl., 38 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9954732	A1	19991028	WO 1999-US8366	19990416
W: AU, CA, JP				
RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				
US 6037133	A	20000314	US 1998-62070	19980417
CA 2326670	AA	19991028	CA 1999-2326670	19990416
AU 9936481	A1	19991108	AU 1999-36481	19990416
AU 747913	B2	20020530		
EP 1071952	A1	20010131	EP 1999-918609	19990416
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI				
US 6093808	A	20000725	US 1999-354183	19990716

PRIORITY APPLN. INFO.: US 1998-62070 A 19980417
WO 1999-US8366 W 19990416

AB The invention presents a **fusion protein** comprising IκB fused to humanized/ **enhanced green fluorescent protein** (**EGFP**). The invention provides an isolated DNA mol. encoding the IκB- **EGFP** **fusion protein**, a vector capable of expressing the DNA mol. and a cell line transfected with said vector. The invention further

presents the use of the IκB- **EGFP** DNA mol. in identifying compds. that activate NFκB. The method involves contacting the IκB- **EGFP** transformed cell with a compound of interest and measuring the amount of **GFP**-induced fluorescence, wherein a decrease in **GFP**-induced fluorescence by said compound indicates that NFκB has been activated by the compound In addition, the decrease in **GFP**-induced fluorescence is directly proportional to the degradation of IκB. The method for identifying compds. that activate NFκB utilizes the fact that NFκB is present in an inactive form in the cytoplasm bound to IκB. Cellular activation in response to a variety of inducers leads to release of NFκB from IκB, followed by IκB being phosphorylated and degraded rapidly by 26S proteasome. Hence the IκB- **EGFP** is used as a tool for measuring the degradation of IκB, which directly correlates with the activation of NFκB. The invention presented **EGFP** as a mutant **GFP** (Phe→Leu at position 64 and Ser→Thr at position 64) encoded by a gene with optimized human codons that has a 35-fold increase in fluorescence.

REFERENCE COUNT: 4 THERE ARE 4 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L8 ANSWER 20 OF 24 MEDLINE on STN DUPLICATE 9

ACCESSION NUMBER: 1999340063 MEDLINE

DOCUMENT NUMBER: PubMed ID: 10409681

TITLE: Characterization of NFkappaB activation by detection of **green fluorescent protein** -tagged IkappaB degradation in living cells.

AUTHOR: **Li X**; Fang Y; Zhao X; Jiang X; Duong T; **Kain S R**

CORPORATE SOURCE: CLONTECH Laboratories, Inc., Palo Alto, California 94303, USA.. xqli@clontech.com

CONTRACT NUMBER: 1R43GM58288-01 (NIGMS)

SOURCE: Journal of biological chemistry, (1999 Jul 23) 274 (30) 21244-50.
Journal code: 2985121R. ISSN: 0021-9258.

PUB. COUNTRY: United States

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English

FILE SEGMENT: Priority Journals

ENTRY MONTH: 199908

ENTRY DATE: Entered STN: 19990910
Last Updated on STN: 20000303
Entered Medline: 19990826

AB Activation of the transcription factor NFkappaB requires rapid degradation of its inhibitor, IkappaBalpha. To facilitate the study of IkappaBalpha degradation, we fused IkappaBalpha protein to **enhanced green fluorescent protein** to construct IkappaBalpha-**enhanced green fluorescent protein** (IG). We demonstrated by both flow cytometry and Western blot analysis that the half-life of IG in the presence of human tumor necrosis factor (TNF) alpha is approximately 5 min, which is similar to the half-life of native IkappaBalpha. The degradation coincided with NFkappaB translocation from the cytoplasm to the nucleus and NFkappaB-mediated induction of transcription. Phorbol 12-myristate 13-acetate (PMA), but not forskolin, also induces degradation of IG **fusion protein**. The half-life of IG in the presence of PMA is approximately 15 min, longer than when induced with TNFalpha. Co-treatment with TNFalpha and PMA did not result in a synergistic effect on IG degradation, although they stimulate different kinases in two different signaling pathways. Degradation of IG was inhibited by mutations at serine residues 32 and 36, which are the target sites of the phosphorylation modification that initiates degradation of IkappaBalpha. We also demonstrated that basal degradation of IG in the presence of cycloheximide is inhibited by such mutations, suggesting that basal

degradation of IkappaBalpha also requires phosphorylation as the signal for degradation. Finally, we showed that the rate of TNFalpha-induced degradation of IG remains almost constant throughout the cell cycle, except at the mitotic phase, in which IG degrades more slowly.

L8 ANSWER 21 OF 24 CAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 2000:62883 CAPLUS
DOCUMENT NUMBER: 132:74557
TITLE: Recombinant plasmid vectors expressing **green fluorescent protein** and hepatitis antigens for immunoassay
INVENTOR(S): Qi, Yipeng; Huang, Yongxiu; Yue, Lili; Deng, Yuanhui; Lu, Liqun; Wang, Yefu; **Li, Xiaofeng**; Lin, Hong; Xiao, Gengfu
PATENT ASSIGNEE(S): Wuhan Univ., Peop. Rep. China
SOURCE: Faming Zhuanli Shenqing Gongkai Shuomingshu, 5 pp.
CODEN: CNXXEV
DOCUMENT TYPE: Patent
LANGUAGE: Chinese
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
CN 1175639	A	19980311	CN 1996-119614	19960901

PRIORITY APPLN. INFO.: CN 1996-119614 19960901
AB Plasmid vector pS65TP-He encoding a **fusion protein** of hepatitis B virus antigen HBeAg with **green fluorescent protein** (**GFP**) of Aequoria victoria and plasmid vector pGC-220 encoding a **fusion protein** of hepatitis C virus core antigen and **GFP** are provided for immunoassay of the antibodies to the viruses. The fusion proteins expressed from the plasmids can be used for rapid diagnosis of human HCV and HBV infection.

L8 ANSWER 22 OF 24 MEDLINE on STN DUPLICATE 10
ACCESSION NUMBER: 1999074273 MEDLINE
DOCUMENT NUMBER: PubMed ID: 9857028
TITLE: Generation of destabilized **green fluorescent protein** as a transcription reporter.
AUTHOR: **Li X**; Zhao X; Fang Y; Jiang X; Duong T; Fan C; Huang C C; **Kain S R**
CORPORATE SOURCE: Laboratories, Inc., Palo Alto, California 94303, USA.. xqli@clontech.com
SOURCE: Journal of biological chemistry, (1998 Dec 25) 273 (52) 34970-5.
Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199902
ENTRY DATE: Entered STN: 19990216
Last Updated on STN: 19990216
Entered Medline: 19990203
AB The **green fluorescent protein** (**GFP**) is a widely used reporter in gene expression and protein localization studies. **GFP** is a stable protein; this property allows its accumulation and easy detection in cells. However, this stability also limits its application in studies that require rapid reporter turnover. We created a destabilized **GFP** for use in such studies by fusing amino acids 422-461 of the degradation domain of mouse ornithine decarboxylase (MODC) to the C-terminal end of an enhanced variant of **GFP** (**EGFP**). The **fusion protein**,

unlike **EGFP**, was unstable in the presence of cycloheximide and had a fluorescence half-life of 2 h. Western blot analysis indicated that the fluorescence decay of **EGFP**-MODC-(422-461) was correlated with degradation of the **fusion protein**. We mutated key amino acids in the PEST sequence of **EGFP**-MODC-(422-461) and identified several mutants with variable half-lives. The suitability of destabilized **EGFP** as a transcription reporter was tested by linking it to NFkappaB binding sequences and monitoring tumor necrosis factor alpha-mediated NFkappaB activation. We obtained time course induction and dose response kinetics similar to secreted alkaline phosphatase obtained in transfected cells. This result did not occur when unmodified **EGFP** was used as the reporter. Because of its autofluorescence, destabilized **EGFP** can be used to directly correlate gene induction with biochemical change, such as NFkappaB translocation to the nucleus.

L8 ANSWER 23 OF 24 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 1997:276650 BIOSIS
DOCUMENT NUMBER: PREV199799575853
TITLE: Expression and detection of **green fluorescent protein** (**GFP**).
AUTHOR(S): **Kain, Steven R.** [Reprint author]; Kitts, Paul
CORPORATE SOURCE: CLONTECH Lab. Inc., Palo Alto, CA, USA
SOURCE: Tuan, R. S. [Editor]. METH MOL BIOL, (1997) pp. 305-324. Methods in Molecular Biology; Recombinant protein protocols: Detection and isolation. Publisher: Humana Press Inc., Suite 808, 999 Riverview Drive, Totowa, New Jersey 07512, USA. Series: Methods in Molecular Biology.
CODEN: MMBYBO. ISSN: 0097-0816. ISBN: 0-89603-400-3 (paper), 0-89603-481-X (cloth).
DOCUMENT TYPE: Book; (Book Chapter)
LANGUAGE: English
ENTRY DATE: Entered STN: 3 Jul 1997
Last Updated on STN: 3 Jul 1997

L8 ANSWER 24 OF 24 MEDLINE on STN DUPLICATE 11
ACCESSION NUMBER: 96113688 MEDLINE
DOCUMENT NUMBER: PubMed ID: 8777060
TITLE: **Green fluorescent protein** as a reporter of gene expression and protein localization.
AUTHOR: **Kain S R**; Adams M; Kondepudi A; Yang T T; Ward W W; Kitts P
CORPORATE SOURCE: CLONTECH Laboratories, Inc., Palo Alto, CA, USA.
SOURCE: BioTechniques, (1995 Oct) 19 (4) 650-5.
Journal code: 8306785. ISSN: 0736-6205.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199609
ENTRY DATE: Entered STN: 19960924
Last Updated on STN: 19980206
Entered Medline: 19960916

AB The **green fluorescent protein** (**GFP**) from the jellyfish Aequorea victoria is rapidly becoming an important reporter molecule for monitoring gene expression and protein localization in vivo, in situ and in real time. **GFP** emits bright green light (lambda max = 509 nm) when excited with UV or blue light (lambda max = 395 nm, minor peak at 470 nm). The fluorescence excitation and emission spectra of **GFP** are similar to those of fluorescein, and the conditions used to visualize this fluorophore are also suitable for **GFP**. Unlike other bioluminescent reporters, the chromophore in **GFP** is intrinsic to the primary structure of the protein, and

GFP fluorescence does not require a substrate or cofactor. **GFP** fluorescence is stable, species-independent and can be monitored non-invasively in living cells and, in the case of transparent organisms, whole animals. Here we demonstrate **GFP** fluorescence in bacterial and mammalian cells and introduce our Living Colors line of **GFP** reporter vectors, **GFP** protein and anti-**GFP** antiserum. The reporter vectors for **GFP** include a promoterless **GFP** vector for monitoring the expression of cloned promoters/enhancers in mammalian cells and a series of six vectors for creating **fusion protein** to either the N or C terminus of **GFP**.

```
=> d his

     (FILE 'HOME' ENTERED AT 16:42:26 ON 09 JUN 2004)

     FILE 'MEDLINE, BIOTECHDS, EMBASE, BIOSIS, SCISEARCH, CANCERLIT, CAPLUS'
     ENTERED AT 16:42:36 ON 09 JUN 2004
L1         53940 S LI X?/AU OR KAIN S?/AU
L2          7357 S FUSION PROTEIN  AND (FLUORESCENT PROTEIN)
L3          9189 S FUSION PROTEIN AND (ENHANC## GREEN FLUORESCENT PROTEIN OR GFP
L4          1417 S FUSION PROTEIN AND (ECFP OR EYFP OR EGFP)
L5            66 S  (L2 OR L3 OR L4) AND HALF LIFE
L6            25 DUP REM L5 (41 DUPLICATES REMOVED)
L7            53 S L1 AND (L2 OR L3 OR L4)
L8            24 DUP REM L7 (29 DUPLICATES REMOVED)

=> s l8 and (half life or half-life)
L9             4 L8 AND (HALF LIFE OR HALF-LIFE)

=> d ibib abs l9 1-4

L9   ANSWER 1 OF 4      MEDLINE on STN
ACCESSION NUMBER:    1999340063      MEDLINE
DOCUMENT NUMBER:     PubMed ID: 10409681
TITLE:               Characterization of NFkappaB activation by detection of
                     green fluorescent protein
                     -tagged IkappaB degradation in living cells.
AUTHOR:              Li X; Fang Y; Zhao X; Jiang X; Duong T; Kain
                     S R
CORPORATE SOURCE:    CLONTECH Laboratories, Inc., Palo Alto, California 94303,
                     USA.. xqli@clontech.com
CONTRACT NUMBER:     1R43GM58288-01 (NIGMS)
SOURCE:              Journal of biological chemistry, (1999 Jul 23) 274 (30)
                     21244-50.
                     Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY:        United States
DOCUMENT TYPE:       Journal; Article; (JOURNAL ARTICLE)
LANGUAGE:            English
FILE SEGMENT:        Priority Journals
ENTRY MONTH:         199908
ENTRY DATE:          Entered STN: 19990910
                     Last Updated on STN: 20000303
                     Entered Medline: 19990826
AB   Activation of the transcription factor NFkappaB requires rapid degradation
     of its inhibitor, IkappaBalpha. To facilitate the study of IkappaBalpha
     degradation, we fused IkappaBalpha protein to enhanced
     green fluorescent protein to construct
     IkappaBalpha-enhanced green fluorescent
     protein (IG). We demonstrated by both flow cytometry and Western
     blot analysis that the half-life of IG in the presence
     of human tumor necrosis factor (TNF) alpha is approximately 5 min, which
     is similar to the half-life of native IkappaBalpha.
```

The degradation coincided with NFkappaB translocation from the cytoplasm to the nucleus and NFkappaB-mediated induction of transcription. Phorbol 12-myristate 13-acetate (PMA), but not forskolin, also induces degradation of IG **fusion protein**. The **half-life** of IG in the presence of PMA is approximately 15 min, longer than when induced with TNFalpha. Co-treatment with TNFalpha and PMA did not result in a synergistic effect on IG degradation, although they stimulate different kinases in two different signaling pathways. Degradation of IG was inhibited by mutations at serine residues 32 and 36, which are the target sites of the phosphorylation modification that initiates degradation of IkappaBalpha. We also demonstrated that basal degradation of IG in the presence of cycloheximide is inhibited by such mutations, suggesting that basal degradation of IkappaBalpha also requires phosphorylation as the signal for degradation. Finally, we showed that the rate of TNFalpha-induced degradation of IG remains almost constant throughout the cell cycle, except at the mitotic phase, in which IG degrades more slowly.

L9 ANSWER 2 OF 4 MEDLINE on STN
ACCESSION NUMBER: 1999074273 MEDLINE
DOCUMENT NUMBER: PubMed ID: 9857028
TITLE: Generation of destabilized **green fluorescent protein** as a transcription reporter.
AUTHOR: **Li X**; Zhao X; Fang Y; Jiang X; Duong T; Fan C; Huang C C; **Kain S R**
CORPORATE SOURCE: Laboratories, Inc., Palo Alto, California 94303, USA.. xqli@clontech.com
SOURCE: Journal of biological chemistry, (1998 Dec 25) 273 (52) 34970-5.
Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199902
ENTRY DATE: Entered STN: 19990216
Last Updated on STN: 19990216
Entered Medline: 19990203

AB The **green fluorescent protein** (**GFP**) is a widely used reporter in gene expression and protein localization studies. **GFP** is a stable protein; this property allows its accumulation and easy detection in cells. However, this stability also limits its application in studies that require rapid reporter turnover. We created a destabilized **GFP** for use in such studies by fusing amino acids 422-461 of the degradation domain of mouse ornithine decarboxylase (MODC) to the C-terminal end of an enhanced variant of **GFP** (**EGFP**). The **fusion protein**, unlike **EGFP**, was unstable in the presence of cycloheximide and had a fluorescence **half-life** of 2 h. Western blot analysis indicated that the fluorescence decay of **EGFP**-MODC-(422-461) was correlated with degradation of the **fusion protein**. We mutated key amino acids in the PEST sequence of **EGFP**-MODC-(422-461) and identified several mutants with variable half-lives. The suitability of destabilized **EGFP** as a transcription reporter was tested by linking it to NFkappaB binding sequences and monitoring tumor necrosis factor alpha-mediated NFkappaB activation. We obtained time course induction and dose response kinetics similar to secreted alkaline phosphatase obtained in transfected cells. This result did not occur when unmodified **EGFP** was used as the reporter. Because of its autofluorescence, destabilized **EGFP** can be used to directly correlate gene induction with biochemical change, such as NFkappaB translocation to the nucleus.

L9 ANSWER 3 OF 4 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
ACCESSION NUMBER: 2001-01398 BIOTECHDS
TITLE: New rapidly degrading **green fluorescent protein** (**GFP**)-fusion proteins, useful in a variety of research applications, e.g. as a reporter in determining gene expression and protein localization; constructed by of fusing C-terminus amino acids of the degradation domain mouse ornithine-decarboxylase
AUTHOR: **Li X**; **Kain S**
PATENT ASSIGNEE: Clontech-Lab.
LOCATION: Palo Alto, CA, USA.
PATENT INFO: US 6130313 10 Oct 2000
APPLICATION INFO: US 1998-62102 17 Apr 1998
PRIORITY INFO: US 1998-62102 17 Apr 1998
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2000-655680 [63]
AN 2001-01398 BIOTECHDS
AB A **fusion protein** comprising **green fluorescent protein** (**GFP**) and a PEST sequence is claimed. The protein has a **half-life** of no more than ten hours. The **fusion protein** is useful in research applications, e.g. as a genetic reporter for analyzing transcriptional regulation and/or cis-acting regulatory element, as a tool for identifying and dissecting degradation domains of short-lived proteins, or in drug screening assays. The method provides a rapid turnover or destabilized **GFP** that can be used in research applications where prior method GFPs cannot. A rapid turnover **GFP** permits development of a stable cell line, which expresses the **GFP** gene since toxic levels of **GFP** are avoided, as the **GFP** protein is quickly degraded. Also disclosed are: an isolated DNA molecule encoding a **GFP fusion protein** with a **half life** decreased from that of wild type **GFP**; a vector capable of expressing the isolated DNA molecule; a method of producing a stable cell line that expresses **GFP** by transfecting cells with the vector; and a method of labelling cells with a transient **GFP** reporter. (18pp)

L9 ANSWER 4 OF 4 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
ACCESSION NUMBER: 2000-01528 BIOTECHDS
TITLE: New **fusion protein** useful for studying cell localization, for studying cell lineage and for assaying activation or deactivation of transcriptional or translational elements; **green fluorescent protein** and mouse ornithine-decarboxylase **fusion protein** expression in host cell
AUTHOR: **Li X**; **Kain S**
PATENT ASSIGNEE: Clontech-Lab.
LOCATION: Palo Alto, CA, USA.
PATENT INFO: WO 9954348 28 Oct 1999
APPLICATION INFO: WO 1998-US24323 13 Nov 1998
PRIORITY INFO: US 1998-62102 17 Apr 1998
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2000-013225 [01]
AN 2000-01528 BIOTECHDS
AB A **fusion protein** containing a **fluorescent protein** related to **green fluorescent protein** and mouse ornithine-decraboxylase (EC-4.1.1.17) degradation domain C-terminal amino acids and having a **half-life** of no more than 10 hr, is new. Also claimed are: a DNA molecule encoding the **fusion protein**; a vector; a stable cell line that expresses a **fluorescent protein**

; and production of the cell line. The protein can be used for assaying activation or deactivation of transcriptional or translational elements with a transient fluorescent reporter protein. The protein can be used to study cell lineage. It can also be linked with different enhancer elements and used to monitor biological processes e.g. heat response, response to cyclic-AMP etc. (49pp)